THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellant(s):	Breton, et al.
Appl. No.:	10/505,305
Conf. No.:	6006
Filed:	October 27, 2004
Title:	ORALLY ADMINISTRABLE COMPOSITION FOR THE PHOTOPROTECTION OF THE SKIN
Art Unit:	1651
Examiner:	Irene Marx
Docket No.:	112701-434

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANTS' APPEAL BRIEF

Sir:

Appellants submit this Appeal Brief in support of the Notice of Appeal filed on January 8, 2009. This Appeal is taken from the Final Rejection in the Office Action dated December 24, 2008.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec S.A., by virtue of an Assignment recorded on October 27, 2004 at reel 015297, frames 0096-0098 in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellant's legal representative and the Assignees of the this patent application do not know of any prior or pending appeals, interferences or judicial proceedings that may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF CLAIMS

Claims 1-10 and 25-30 are pending in this application. Claims 11-24 were previously canceled. Claims 1-10 and 25-30 stand rejected. Therefore, Claims 1-10 and 25-30 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

IV. STATUS OF AMENDMENTS

A non-final Office Action was mailed on July 24, 2008 rejecting the claims as obvious in view of several cited references. Appellants responded to the non-final Office Action on October 23, 2008 without amending the claims to overcome the obvious rejection set forth in the non-final Office Action. A final Office Action maintaining the rejections was mailed on December 24, 2008. Appellants filed a Notice of Appeal on January 8, 2009. A copy of the non-final Office Action and final Office Action are attached as Exhibits A and B, respectively, in the Evidence Appendix.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the claimed subject matter by way of reference to the specification and/or figures for each of the independent claims is provided as follows:

Independent Claim 1 is directed to an orally administrable composition for the photoprotection of the skin (page 2, lines 18-29) comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium (page 2, lines 18-29; page 3, line 18 to page 5, line 15; Examples 1-6), and at least one carotenoid (page 2, lines 18-29; page 3, lines 18-24; page 5, lines 17-24; Examples 1-6), wherein the at least one carotenoid is present in the composition in an amount from 10^{-12}% to 20% by weight (page 5, lines 17-24), included in an orally acceptable carrier (page 5, line 28 to page 6, line 6), the composition further comprising a yeast extract (page 6, lines 8-20).

Although specification citations are given in accordance with 37 C.F.R. §1.192(c), these reference numerals and citations are merely examples of support in the specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the references numerals and citations, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology in accordance with Rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the specification. In short, the references numerals and specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1-10 and 25-30 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over EP Patent No. 1020123 to Cavaliere Vesely, et al. ("*Vesely*") taken with U.S. Patent No. 6,156,355 to Shields, Jr., et al. ("*Shields*"), U.S. Patent No. 7,037,708 to Runge, et al. ("*Runge*"), WO 00/79072 to Berggren, et al. ("*Berggren*") and U.S. Patent No. 5,603,930 to Brassart, et al. ("*Brassart*") and further taken with U.S. Patent No. 4,806,368 to Reddy ("*Reddy*"). Copies of *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy* are attached hereto as Exhibits C, D, E, F, G and H, respectively, in the Evidence Appendix.

VII. ARGUMENT

A. LEGAL STANDARDS

Obviousness under 35 U.S.C. §103

The Federal Circuit has held that the legal basis for a determination of obviousness under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Examiner has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 U.S.P.Q. 2d 1955, 1956 (Fed. Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

Moreover, the Examiner must provide explicit reasons why the claimed invention is obvious in view of the prior art. The Supreme Court has emphasized that when formulating a rejection under 35 U.S.C. § 103(a) based upon a combination of prior art elements it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed. *KSR v. Teleflex*, 127 S. Ct. 1727 (2007).

Of course, references must be considered as a whole and those portions teaching against or away from the claimed invention must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered to teach away when a person of ordinary skill, upon reading the reference would be discouraged

from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Mach. Corp. v. Fukuhara Indus. Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998) (quoting *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994)).

B. THE CLAIMED INVENTION

There are one independent claim on appeal: Claim 1. Independent Claim 1 is generally directed to an orally administrable composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, and at least one carotenoid. The one or more carotenoids are present in the composition in an amount ranging from 10^{-12}% to 20% by weight and are included in an orally acceptable carrier. The composition further comprises a yeast extract.

C. THE REJECTION OF CLAIMS 1-10 AND 25-30 UNDER 35 U.S.C. §103(A) TO *VESELY, SHIELDS, RUNGE, BERGGREN, BRASSART* AND *REDDY* SHOULD BE REVERSED BECAUSE THE EXAMINER HAS FAILED TO ESTABLISH A *PRIMA FACIE* CASE OF OBVIOUSNESS WITH RESPECT TO CLAIMS 1-10 AND 25-30

1. *Vesely, Shields, Runge, Berggren, Brassart* and *Reddy* alone or in combination fail to disclose or suggest the claimed invention

Independent Claim 1 recites an orally administrable composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, and at least one carotenoid or derivative present in the composition in an amount from 10^{-12}% to 20% by weight, and a yeast extract. The presently claimed oral composition includes an admixture of specific constituents that surprisingly and unexpectedly elicit an enhanced effect or response with respect to the photoprotection of the skin. See specification, page 14, lines 6-11. Moreover, the skilled artisan would understand that the probiotic lactic acid bacterium,

carotenoid and yeast extract are combined together in a single orally acceptable carrier. In contrast, Appellants respectfully submit that *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy* alone or in combination fail to disclose or suggest a single orally acceptable carrier comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract as required by independent Claim 1.

Vesely is directed toward a beverage containing live bacteria that is used to increase, balance and supplement intestinal flora. See *Vesely*, column 3, paragraph 0016. *Vesely* fails to explicitly teach or show any examples of an orally acceptable carrier having a probiotic lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1. In fact, *Vesely* fails to mention a yeast extract anywhere in its disclosure.

Shields is directed toward canine food formulations that optimize digestibility of nutrients in specific canine breeds. See *Shields*, column 3, lines 30-36. *Shields* fails to explicitly teach or show any examples of an orally acceptable carrier having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1. Nowhere does *Shields* teach or even suggest a photoprotecting effective amount of at least one probiotic lactic acid bacterium. Rather, *Shields* discloses pet food formulations that generally list at least 50 or more ingredients without specifying amounts for each ingredient. See *Shields*, Examples 1-7.

Runge is directed toward dry microorganism cultures and the processes for producing same. See *Runge*, Abstract. *Runge* fails to explicitly teach or show any examples of an orally acceptable carrier having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1.

Berggren is directed toward a sports drink that is designed to increase the energy and fluid levels in an individual as well as reduce stress. See *Berggren*, page 2, line 39-page 3, line 4. *Berggren* fails to explicitly teach or show any examples of an orally acceptable carrier having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1. In this regard, *Berggren* fails to mention a yeast extract anywhere in its disclosure.

Brassart is directed toward a biologically pure culture of a lactic acid bacterium strain. See *Brassart*, Summary of the Invention. *Brassart* fails to explicitly teach or show any examples of an orally acceptable carrier having a photoprotecting effective amount of at least one probiotic

lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1. In fact, *Brassart* fails to mention a carotenoid or yeast extract anywhere in its disclosure.

Reddy is directed toward a supplement that permits the longevity of certain health promoting bacteria in tablets. See *Reddy*, column 1, lines 10-20. *Reddy* fails to explicitly teach or show any examples of an orally acceptable carrier having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract combined together in accordance with Claim 1.

For at least the reasons discussed above, *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy* alone or in combination fail to disclose or suggest every element of independent Claim 1. Accordingly, Appellants respectfully submit that Claim 1, along with Claims 2-10 and 25-30 that depend from Claim 1, are novel, nonobvious and distinguishable from the cited references and are in condition for allowance.

2. The skilled artisan would have no reason to combine *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy* to arrive at the claimed invention

Appellants respectfully submit that the skilled artisan would not arrive at the claimed invention using the cited references in the absence of hindsight because the cited references are entirely directed to compositions utilizing different nutritional ingredients for different intended purposes. Moreover, Appellants respectfully submit that the Examiner is using Appellants' patent application as a road map for creating hindsight obviousness and has failed to set forth sufficient reasons for how the skilled artisan would arrive at the claimed invention in view of *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy*.

Appellants previously submitted an Affidavit under 37 C.F.R. §1.132 ("*Affidavit*" attached hereto as Exhibit I) that demonstrates the deficiencies of the prior art with respect to the present claims. The *Affidavit* summarizes a controlled study performed by Appellants that demonstrates the surprising and unexpected synergistic photoprotective effects resulting from ingestion of the presently claimed composition comprising an admixture of a photoprotecting effective amount of at least one probiotic lactic acid bacterium, at least one carotenoid, and a

yeast extract. As demonstrated by the study discussed in the *Affidavit*, the presently claimed composition has been found to be effective not only for preventing inflammation or irritation of the skin after exposure to ultraviolet radiation ("UVR"), but it has also been found effective to provide complete prophylactic protection against the immunosuppressive effects of ultraviolet radiation. Specifically, the composition of the present disclosure is able to block or reduce the adverse clinical, histological and immunological effects of solar radiation exposure on the skin.

As further supported by the study and *Affidavit*, it is the specific combination of the probiotic lactic acid bacterium, the carotenoid, and the yeast extract that provides the surprising and unexpected synergistic photoprotective effects on the skin. For example, as illustrated by Figure 2 of Exhibit B, the control test without UVR exposure (column 2) and the composition according to the present disclosure and having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, at least one carotenoid, and a yeast extract (column 3) showed the greatest immunological response to the dinitroflurobenzene ("DNFB") allergen. This was demonstrated by the larger differences between the swelling of the right and left ears of the mice. The increased amount of swelling of the right ear of the mice tested with respect to columns 2 and 3 indicated that the skin reacted readily to the presence of the allergen on the right ear. In other words, the skin reacted readily to the presence of the allergen on the right ear because the animal did not experience local immunosuppression due to exposure to UVR.

As further illustrated by Figure 2 of Exhibit B, the control test plus exposure to UVR (column 1), the "matrix" formula (i.e. carotenoid and yeast with no probiotic) plus exposure to UVR (column 4), and the carotenoids alone plus exposure to UVR (column 5) all failed to block or reduce the clinical, histological and immunological effect of UVR exposure of the skin of the animal. These results were clearly demonstrated by the decreased amount of swelling of the right ear of the animal, which indicated that the immune system of the animal was not acting efficiently because the immune system was suppressed by the exposure to UVR. In other words, the formulas corresponding with the data of columns 1 and 4-5 proved unsuccessful in preventing local immunosuppression resulting from UVR exposure.

Appellants respectfully submit that the *Affidavit* and the study discussed therein clearly demonstrates the synergistic photoprotective effects on the skin of an animal that has ingested the presently claimed composition comprising a photoprotecting effective amount of probiotic lactic acid bacterium, a carotenoid and a yeast extract. The effects of the presently claimed

composition have been compared in the study set forth in the *Affidavit* to compositions having i) only carotenoids and yeast, and ii) carotenoids alone. As such, the study and *Affidavit* clearly demonstrate the efficacy of the presently claimed composition and the importance of the combination of all three components including the probiotic lactic acid bacterium, a carotenoid and a yeast extract.

In view of the specification and the *Affidavit*, Appellants respectfully submit that the skilled artisan would have no reason to combine the cited references to obtain the present claims because the cited references are directed to unrelated products that have completely different objectives. Moreover, the cited references fail to even recognize the surprising and unexpected effects that the specifically claimed combination of components in the orally administrable composition has on the photoprotection of the skin.

Vesely is entirely directed toward a beverage containing live bacteria that is used to increase, balance and supplement intestinal flora. See *Vesely*, column 3, paragraph 0016. *Vesely* teaches that the beverage can contain other general ingredients such as vitamins, minerals, sugars and flavors. Nevertheless, *Vesely* fails to teach that any of these additional ingredients are essential to achieve his objectives and further fails to teach any yeast extracts. As a result, *Vesely* fails to teach or provide any guidance to the skilled artisan regarding a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

Shields is entirely directed toward canine food formulations that optimize digestibility of nutrients in specific canine breeds. See *Shields*, column 3, lines 30-36. *Shields* teaches that these canine food formulations include numerous ingredients including meet, rice, antioxidants, fats, organic minerals, fibers, herbs and spices and lists specific examples of formulations that include 50 or more of these ingredients. See *Shields*, Examples 1-7. This general recitation of ingredients by *Shields* fails to teach or provide any guidance to the skilled artisan regarding a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

Runge is entirely directed toward dry microorganism cultures and the processes for producing same. See *Runge*, Abstract. The focus of *Runge* is preparing dry microorganism cultures so that they can be advantageously added to foodstuffs and feedstuffs. See *Runge*,

column 2, line 32 to column 3, line 29. *Runge* fails to teach or provide any guidance to the skilled artisan regarding a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

Berggren is entirely directed toward a sports drink that is designed to increase the energy and fluid levels in an individual as well as reduce stress. See *Berggren*, page 2, line 39-page 3, line 4. *Berggren* generally recites a numerous list of components including vitamins and minerals but does not recite any yeast extract. Accordingly, *Berggren* fails to teach or provide any guidance to the skilled artisan regarding a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

Brassart is entirely directed toward a biologically pure culture of a lactic acid bacterium strain. See *Brassart*, Summary of the Invention. In fact, *Brassart* fails to recite any additional nutritional ingredients such as carotenoids or yeast extracts for combining with the lactic acid bacterium strain and therefore fails to teach or provide any guidance to the skilled artisan regarding a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

Reddy is entirely directed toward a supplement that permits the longevity of certain health promoting bacteria in tablets. See *Reddy*, column 1, lines 10-20. *Reddy* is unconcerned with the photoprotection of skin. As a result, *Reddy* fails to teach or provide any guidance to the skilled artisan how to make a single orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1.

The Examiner asserts that there is no nexus or correlation between the invention as claimed and the results provided. See final Office Action, page 4. More specifically, the Examiner asserts that there is no nexus or correlation between the live Lal of the *Affidavit* and the invention as claimed directed to any probiotic acid bacterium and the various species and strains of Claims 3 and 4, which do not appear to include an amount of live Lal. Appellants respectfully disagree. As known by persons skilled in the art, "Lal" is an abbreviation of

Lactobacillus johnsonii. Accordingly, *Lactobacillus johnsonii* is clearly recited in Claim 3 as an example of the probiotic lactic acid bacterium generally recited in independent Claim 1.

The Examiner also asserts that the *Affidavit* does not clearly distinguish over the art and is not probative of unexpected results since it is not commensurate in scope with the claims. Appellants respectfully disagree with this assertion and submit that the showing of the *Affidavit* is commensurate with the scope of the claims by establishing, as stated above, that the control test without UVR exposure (column 2) and the composition according to the present disclosure and having a photoprotecting effective amount of at least one probiotic lactic acid bacterium (e.g., La1 – *Lactobacillus johnsonii*), at least one carotenoid, and a yeast extract (column 3) showed the greatest immunological response to the DNFB allergen, as is demonstrated by the larger differences between the swelling of the right and left ears of the mice. See *Affidavit*, #11. In this regard, the *Affidavit* demonstrates the photoprotective qualities of the composition recited in the claims.

Moreover, the skilled artisan would understand that broadening out the La1 – *Lactobacillus johnsonii* to encompass other probiotics and broadening out lycopene to encompass other carotenoids serves as Appellants' prophetic examples. A prophetic example describes an embodiment of the invention based on predicted results rather than work actually conducted or results actually achieved.

Appellants respectfully submit that it is only with a hindsight reconstruction of Appellants' claimed invention that the Examiner is able to even attempt to piece together the teachings of the prior art so that the claimed invention is allegedly rendered obvious. However, the claims must be viewed as a whole as defined by the claimed invention and not dissected into discrete elements to be analyzed in isolation. *W.L. Gore & Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1548, 220 USPQ 303, 309 (Fed. Cir. 1983); *In re Ochiai*, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995). One should not use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention. *In re Fine*, 837 F.2d at 1075. (Fed. Cir. 1988). In this regard, Appellants respectfully submit that there is absolutely no guidance in the cited reference for one of skill in the art to choose the active agents and amount of agents present in the instant claims to achieve the unexpectedly improved photoprotective effect on the skin as Appellants have done.

The Examiner assumes that it would have been within the ordinary skill of the artisan at the time the claimed invention was made because the references relied upon allegedly teach that all aspects of the claimed invention were individually known in the art. However, this conclusory statement is not sufficient to establish a *prima facie* case of obviousness without some objective reason to utilize the teachings of the references to arrive at the invention. *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993). There must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness by the Examiner. *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006).

To arrive at the claimed combinations of a photoprotecting effective amount of at least one probiotic lactic acid bacterium, a carotenoid and a yeast extract in accordance with Claim 1, the skilled artisan would have to select these three specific components from the hundreds of nutritional ingredients taught by the cited references and then combine them in the specified amounts in a single orally administrable formulation. Moreover, the cited references do not provide any direction or guidance to select and combine these three specific components from the large number of possible combinations, especially given that they are sometimes non-essential (e.g. optional) ingredients to the nutritional formulations taught by *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy*.

In sum, the skilled artisan would have no reason to arrive at the claimed invention using the cited references in the absence of hindsight. Moreover, *Vesely*, *Shields*, *Runge*, *Berggren*, *Brassart* and *Reddy* fail to even recognize the advantages, benefits and/or properties of an orally administrable composition for the photoprotection of the skin in accordance with the present claims. Instead, Appellants respectfully submit that the Examiner is improperly using Appellants' patent application as a road map for creating hindsight obviousness. Accordingly, Appellants respectfully submit that Claim 1, along with Claims 2-10 and 25-30 that depend from Claim 1, are novel, nonobvious and distinguishable from the cited references and are in condition for allowance.

VIII. CONCLUSION

Appellants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. §103 with respect to the rejection of Claims 1-10 and 25-30. Accordingly, Appellants respectfully submit that the obviousness rejection is erroneous in law and in fact and should therefore be reversed by this Board.

A check in the amount of $510 is submitted herewith to cover the cost of the Appeal Brief. The Director is authorized to charge any additional fees that may be required, or to credit any overpayment to Deposit Account No. 02-1818. If such a withdrawal is made, please indicate the Attorney Docket No. 112701-434 on the account statement.

Respectfully submitted,

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BY 

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Dated: March 3, 2009

CLAIMS APPENDIX

PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 10/505,305

1. An orally administrable composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, and at least one carotenoid, wherein the at least one carotenoid is present in the composition in an amount from 10^{-12}% to 20% by weight, included in an orally acceptable carrier, the composition further comprising a yeast extract.

2. A composition according to claim 1, wherein the lactic acid bacterium is selected from the group consisting of Lactobacilli and Bifidobacteria.

3. A composition according to claim 1, wherein the lactic acid bacterium is selected from the group consisting of *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, and mixtures thereof.

4. A composition according to claim 2, wherein the lactobacilli is selected from the group consisting of *Lactobacillus johnsonii* (CNCM I-1225) and *Lactobacillus paracasei* (CNCM I-2116), and wherein the bifidobacteria is selected from the group consisting of *Bifidobacterium adolescentis* (CNCM I-2168) and *Bifidobacterium longum* (CNCM I-2170).

5. A composition according to claim 1, wherein the probiotic lactic acid bacterium is included in the carrier in a form selected from the group consisting of live form, semi-active and deactivated form.

6. A composition according to claim 1, wherein the carotenoid is selected from the group consisting of a carotenoid with and without provitamin A activity.

7. A composition according to claim 1, wherein the carrier is a food.

8. A composition according to claim 7, wherein the food is selected from the group consisting of milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, and infant formula.

9. A composition according to claim 8, wherein the food is a drinkable solution.

10. A composition according to claim 1, which further comprises a bioactive molecule.

25. A composition according to claim 1, wherein the probiotic lactic acid bacterium is included into the carrier in a lyophilized powder.

26. A composition according to claim 1, wherein the carotenoid is selected from the group consisting of β-carotene, γ-carotene, α-carotene, lycopene, zeaxanthine, luteine, and combinations thereof.

27. A composition according to claim 1, wherein the carrier is a pharmaceutical carrier.

28. A composition according to claim 27, wherein the pharmaceuticals carrier is selected from the group consisting of tablets, liquid suspensions, dried oral supplements, wet oral supplements, and combinations thereof.

29. A composition according to claim 1, wherein the carrier is a nutritional supplement for oral administration.

30. A composition according to claim 29, wherein the nutritional supplement for oral administration is in a form selected from the group consisting of capsules, soft capsules, tablets, pastes, pastilles, gums, drinkable solutions, emulsions, and combinations thereof.

EVIDENCE APPENDIX

EXHIBIT A: Non-final Office Action dated July 24, 2008

EXHIBIT B: Final Office Action dated December 24, 2008

EXHIBIT C: EP Patent No. 1020123 to Cavaliere Vesely, et al. ("*Vesely*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT D: U.S. Patent No. 6,156,355 to Shields, Jr., et al. ("*Shields*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT E: U.S. Patent No. 7,037,708 to Runge, et al. ("*Runge*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT F: WO 00/70972 to Berggren, et al. ("*Berggren*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT G: U.S. Patent No. 5,603,930 to Brassart, et al. ("*Brassart*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT H: U.S. Patent No. 4,806,368 to Reddy ("*Reddy*"), cited by the Examiner in the Office Action dated December 24, 2008

EXHIBIT I: Affidavit under 37 C.F.R. §1.132 to Isabelle Bureau-Franz

RELATED PROCEEDINGS APPENDIX

None

EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/505,305	10/27/2004	Lionel Breton	112701-434	6006

29157 7590 07/24/2008
BELL, BOYD & LLOYD LLP
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NO
References Downloaded
NO

EXAMINER	
MARX, IRENE	
ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
07/24/2008	ELECTRONIC

Date: 10-24-08: NOW FINAL OA

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):
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Office Action Summary	Application No.	Applicant(s)	
	10/505,305	BRETON ET AL.	
	Examiner	Art Unit	
	Irene Marx	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 May 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *11-24* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10 and 25-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/20/08 has been entered.

Claims 1-10 and 25-30 are being examined to the extent that they are directed to a bacterial culture only.

Claims 11-24 are withdrawn from consideration.

The rejection under 35 U.S.C 112, first paragraph regarding deposit is withdrawn in view of applicant's averments..

Claim Rejections - 35 USC § 103

Claims 1-10 and 25-30 are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Cavaliere Vesely *et al.* (EP 1020123) taken with Shields, Jr. *et al.* (U.S. Patent 6,156,355), Runge *et al.* (U.S. Patent No. 7,037,708), Berggren *et al.*. (WO/00/79072) and Brassart *et al.* U.S. Patent No. 5,603,930) and further taken with Reddy (U.S. Patent No. 4,806,368).

The claims are directed to an orally administrable comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium and at least one carotenoid in some amount included in an ingestible carrier and including yeast extract.

As discussed in previous Office actions, the cited references disclose an orally administrable composition including a food or drink composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium and at least one carotenoid, included in an ingestible carrier.

The references differ from the claimed invention in that the specific strains of claim 4 are not disclosed and in the provision of milk products, milk based fermented products such as yogurt.. However, Brassart *et al.* adequately demonstrates that at least strains CNCM I-1225 and CNCM I-1226 are old and well known in the art as probiotics. In addition the reference demonstrates that the composition is provided as a yogurt a milk-based fermented product. See, e.g., col. 3, lines 55-60.

With regard to the pharmaceutical carrier, it is noted that the product of Berggren is provided at least in tablet form. In addition, yogurt containing strain CNCM I-1225 comprises a pharmaceutical carrier.

Regarding the presence of yeast extract, it is noted that Reddy adequately demonstrates that *Lactobacillus* and *Bifidobacterium* compositions containing yeast extract are old and well known in the art. See, e.g., Col. 3, Table. The reference discloses the benefits of the yeast extracts components to the viability of the bacteria, for example as a source of B vitamins.

The concentrations discussed in the references appear to be substantially the same as claimed. However, even if they are not, the adjustment of concentrations for optimization purposes identified as result-effective variables cited in the references would have been prima facie obvious to a person having ordinary skill in the art, since such adjustment is at the essence of biotechnical engineering.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the products of Cavaliere Vesely *et al.*, Shields, Jr. *et al.*, Runge *et al.* and Berggren *et al.* by providing a composition comprising a photoprotective amount of probiotic strains CNCM I-1225 and CNCM I-1226 as suggested by the teachings of Brassart *et al.* for the expected benefit of providing a healthful composition comprising a probiotic strain known to have favorable effects against disease and carotenoids such as β-carotene know to have at least antioxidants effects.

Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

Response to Arguments

Applicant's arguments and Bureau-Franz Declaration have been fully considered but they are not deemed to be persuasive.

The declarant states at 4. that the invention is directed, in part, to an oral composition that includes an admixture of very specific constituents and alleges synergistic effects therefor. However, claim 1 is directed to

"An orally administrable composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, and at least one carotenoid wherein the at least one carotenoid is present in the composition in an amount from

10^{-12}% to 20% by weight, included in an orally acceptable carrier, the composition further comprising a yeast extract".

There is nothing specific about the constituents of this composition considering that the invention as claimed in the independent claim is directed to any probiotic lactic acid bacterium and is directed to various species thereof in claim 3. Only in claim 4 are specific strains claim designated, of which at least two strains are known in the art as shown by Brassart. In addition, the declarant fails to consider that the claim designated invention encompasses unidentified carotenoids at a level of between 10^{-12}% to 20% by weight. The nature of the carotenoids and the amount of 10^{-12}% is not addressed in the declaration and cannot be considered to be "specific". Moreover the compositions comprises an unidentified of extract of unidentified yeasts.

The declaration shows favorable results for a composition comprising "matrix + La1 10^8 live + UV and this is designated "composition according to the invention". In section 8. it is stated that

> "Depending on the group being tested, the mice were fed a variety of formulas of food including a food with no additional supplements; a food with maltodextrin; a "matrix" food having beta-carotene, lycopene, inactivated yeast extract and excipients such as, for example, magnesium stearate, corn starch, and silicon dioxide; a food with carotenoids; and the "matrix" food that was also supplemented with a bacteria (Lal). The formulas for the treatments are set forth in Table 1 of Exhibit B."

There is no clear nexus or correlation between the invention as claimed and the results provided. There is no clear nexus or correlation between the 10^8 live La1 of the declaration and the invention as claimed directed to any probiotic lactic acid bacterium, the various species of claim 3 and even the specific strains of claim 4, which do not appear to include any amount of live "La1", which is not identified with any specificity. In addition, the declaration does not specify the level of carotenoids between 10^{-12}% to 20% by weight in the composition or the amount of yeast extract. It is also noted that claim 5 specifically recites "semi-active" or deactivated" lactic acid bacteria as opposed to "live" as in the declaration.

The Declaration does not clearly distinguish over the art and is not probative of unexpected results since it is not commensurate in scope with the claims. The scope of the showing must be commensurate with the scope of claims to consider evidence probative of

unexpected results, for example. In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980). It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the claim.

In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 19880; In re Jones, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, the Cavaliere Vesely *et al.*, Shields, Jr. *et al.,* Runge *et al.*, Berggren *et al.* are all directed to compositions comprising lactic acid probiotics and carotenoid or carotenoid derivatives even though references do not teach that the composition can be used for photoprotection of the skin. However, the intended use of the composition does not distinguish the composition since such undisclosed use is inherent in the cited compositions. In order to be limiting, the intended use must create a structural difference between the claimed composition and the prior art compositions. In the instant case, the intended use does not create a structural difference, thus, the intended use is not limiting. "The claiming of a new use . . . which is inherently present in the prior art does not necessarily make the claim patentable." *In re Best*, 195 USPQ 430, 433 (CCPA 1977). When applicant claims a "composition in terms of function . . . and the composition of the prior art is the same as that of the claim but the function is not explicitly disclosed by the reference, the Examiner may make a rejection under both 35 U.S.C. 102 and 103, expressed as a 102/103 rejection" (MPEP 2112). In this regard, it is noteworthy that the claims are directed to a composition comprising "10^{-12}% to 20% by weight of an unidentified carotenoid.. There is nothing on the record to indicate to what extent a composition comprising carotenoids at the lower end of the range recited will have the required effect.

As noted previously the addition of yeast extract to probiotic lactic acid compositions is well known in the art for its viability enhancement.

Therefore the rejection is deemed proper and it is adhered to.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Irene Marx whose telephone number is (571) 272-0919. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G. Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 .

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Irene Marx/
Primary Examiner
Art Unit 1651

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10505305	BRETON ET AL.
	Examiner	Art Unit
	Irene Marx	1651

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Search updated	5/6/08	IM
Search updated	7/16/08	IM

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/505,305	10/27/2004	Lionel Breton	112701-434	6006

29157 7590 12/24/2008
BELL, BOYD & LLOYD LLP
P.O. Box 1135
CHICAGO, IL 60690

Final Mailed 3-24-09

EXAMINER	
MARX, IRENE	
ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
12/24/2008	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATENTS@BELLBOYD.COM

Office Action Summary	Application No.	Applicant(s)	
	10/505,305	BRETON ET AL.	
	Examiner	Art Unit	
	Irene Marx	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 October 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-30* is/are pending in the application.

4a) Of the above claim(s) *11-24* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10 and 25-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed 10/23/08 is acknowledged.

Claims 1-10 and 25-30 are being examined to the extent that they are directed to a bacterial culture only.

Claims 11-24 are withdrawn from consideration.

Claim Rejections - 35 USC § 103

Claims 1-10 and 25-30 are/remain rejected under 35 U.S.C. 103(a) as being unpatentable over Cavaliere Vesely *et al.* (EP 1020123) taken with Shields, Jr. *et al.* (U.S. Patent 6,156,355), Runge *et al.* (U.S. Patent No. 7,037,708), Berggren *et al.*. (WO/00/79072) and Brassart *et al.* U.S. Patent No. 5,603,930) and further taken with Reddy (U.S. Patent No. 4,806,368).

The claims are directed to an orally administrable composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium and at least one carotenoid in some amount included in an ingestible carrier and including yeast extract.

As discussed in previous Office actions, the cited references disclose an orally administrable composition including a food or drink composition comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium and at least one carotenoid, included in an ingestible carrier.

The references differ from the claimed invention in that the specific strains of claim 4 are not disclosed and in the provision of milk products, milk based fermented products such as yogurt.. However, Brassart *et al.* adequately demonstrates that at least strains CNCM I-1225 and CNCM I-1226 are old and well known in the art as probiotics. In addition the reference demonstrates that the composition is provided as a yogurt a milk-based fermented product. See, e.g., col. 3, lines 55-60.

With regard to the pharmaceutical carrier, it is noted that the product of Berggren is provided at least in tablet form. In addition, yogurt containing strain CNCM I-1225 comprises a pharmaceutical carrier.

Regarding the presence of yeast extract, it is noted that Reddy adequately demonstrates that *Lactobacillus* and *Bifidobacterium* compositions containing yeast extract are old and well

known in the art. See, e.g., Col. 3, Table. The reference discloses the benefits of the yeast extracts components to the viability of the bacteria, for example as a source of B vitamins.

The concentrations discussed in the references appear to be substantially the same as claimed. However, even if they are not, the adjustment of concentrations for optimization purposes identified as result-effective variables cited in the references would have been prima facie obvious to a person having ordinary skill in the art, since such adjustment is at the essence of biotechnical engineering.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to modify the products of Cavaliere Vesely *et al.*, Shields, Jr. *et al.*, Runge *et al.* and Berggren *et al.* by providing a composition comprising a photoprotective amount of probiotic strains CNCM I-1225 and CNCM I-1226 as suggested by the teachings of Brassart *et al.* for the expected benefit of providing a healthful composition comprising a probiotic strain known to have favorable effects against disease and carotenoids such as β-carotene know to have at least antioxidants effects.

Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

Response to Arguments

Applicant's arguments and Bureau-Franz Declaration have been fully considered but they are not deemed to be persuasive.

The declarant states at 4. that the invention is directed, in part, to an oral composition that includes an admixture of very specific constituents and alleges synergistic effects therefor. However, claim 1 is directed to

"An orally administrable composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, and at least one carotenoid wherein the at least one carotenoid is present in the composition in an amount from 10^{-12}% to 20% by weight, included in an orally acceptable carrier, the composition further comprising a yeast extract".

There is nothing specific about the constituents of this composition. The invention as claimed in the independent claim is directed very broadly to any probiotic lactic acid bacterium and is directed to various species thereof in claim 3. Only in claim 4 are specific strains claim

designated, of which at least two strains are known in the art as shown by Brassart. In addition, the invention as claimed encompasses unidentified carotenoids at a level of between 10^{-12}% to 20% by weight. The nature of the carotenoids and the amount of 10^{-12}% are not addressed in the declaration and cannot be considered to be "specific". Moreover the compositions comprises an unidentified amount of yeast extract from unidentified yeasts.

The declaration shows favorable results for a composition comprising "matrix + La1 10^8 live + UV and this is designated "composition according to the invention". In section 8. it is stated that

> "Depending on the group being tested, the mice were fed a variety of formulas of food including a food with no additional supplements; a food with maltodextrin; a "matrix" food having beta-carotene, lycopene, inactivated yeast extract and excipients such as, for example, magnesium stearate, corn starch, and silicon dioxide; a food with carotenoids; and the "matrix" food that was also supplemented with a bacteria (Lal). The formulas for the treatments are set forth in Table 1 of Exhibit B."

There is no clear nexus or correlation between the invention as claimed and the results provided. There is no clear nexus or correlation between the 10^8 live La1 of the declaration and the invention as claimed directed to any probiotic lactic acid bacterium, the various species of claim 3 and even the specific strains of claim 4, which do not appear to include any amount of live "La1", which is not identified with any specificity. In addition, the declaration does not specify the level of carotenoids between 10^{-12}% to 20% by weight in the composition or the amount of yeast extract. It is also noted that claim 5 specifically recites "semi-active" or deactivated" lactic acid bacteria as opposed to "live" as in the declaration.

The Declaration does not clearly distinguish over the art and is not probative of unexpected results since it is not commensurate in scope with the claims. The scope of the showing must be commensurate with the scope of claims to consider evidence probative of unexpected results, for example. In re Dill, 202 USPQ 805 (CCPA, 1979), In re Lindner 173 USPQ 356 (CCPA 1972), In re Hyson, 172 USPQ 399 (CCPA 1972), In re Boesch, 205 USPQ 215, (CCPA 1980), In re Grasselli, 218 USPQ 769 (Fed. Cir. 1983), In re Clemens, 206 USPQ 289 (CCPA 1980). It should be clear that the probative value of the data is not commensurate in scope with the degree of protection sought by the claim.

In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 19880; In re Jones, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, the Cavaliere Vesely *et al.*, Shields, Jr. *et al.*, Runge *et al.*, Berggren *et al.* are all directed to compositions comprising lactic acid probiotics and carotenoid or carotenoid derivatives even though references do not teach that the composition can be used for photoprotection of the skin. However, the intended use of the composition does not distinguish the composition since such undisclosed use is inherent in the cited compositions. In order to be limiting, the intended use must create a structural difference between the claimed composition and the prior art compositions. In the instant case, the intended use does not create a structural difference, thus, the intended use is not limiting. "The claiming of a new use . . . which is inherently present in the prior art does not necessarily make the claim patentable." *In re Best*, 195 USPQ 430, 433 (CCPA 1977). When applicant claims a "composition in terms of function . . . and the composition of the prior art is the same as that of the claim but the function is not explicitly disclosed by the reference, the Examiner may make a rejection under both 35 U.S.C. 102 and 103, expressed as a 102/103 rejection" (MPEP 2112). In this regard, it is noteworthy that the claims are directed to a composition comprising "10^{-12}% to 20% by weight of an unidentified carotenoid.. There is nothing on the record to indicate to what extent a composition comprising carotenoids at the lower end of the range recited will have the required effect.

As noted previously the addition of yeast extract to probiotic lactic acid compositions is well known in the art for its viability enhancement.

In response to the arguments at page 5, paragraph 3 that the showing of the Affidavit is commensurate with the scope of the claims, it is noted that the use of the strain *Lactobacillus johnsonii* La1 is not clearly claim designated. Moreover, neither the data in the declaration nor the claim designated invention clearly designate the amount and nature of the carotenoids required or the nature or amount of yeast extract provided. Therefore, the Affidavit fails to demonstrate the unexpected photoprotective qualities of the composition **as claimed**.

Therefore the rejection is deemed proper and it is adhered to.

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Irene Marx whose telephone number is (571) 272-0919. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G. Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300 .

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/Irene Marx/
Primary Examiner
Art Unit 1651

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	10505305	BRETON ET AL.
	Examiner	Art Unit
	Irene Marx	1651

SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
Search updated	5/6/08	IM
Search updated	7/16/08	IM
Search updated	12/19/08	IM

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

EXHIBIT C

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 020 123 A1

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
19.07.2000 Bulletin 2000/29

(51) Int Cl.[7]: **A23L 1/03**, A23L 2/52

(21) Application number: **99830013.1**

(22) Date of filing: **18.01.1999**

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
AL LT LV MK RO SI

(71) Applicant: **Sitia-Yomo S.p.A.**
20123 Milano (IT)

(72) Inventors:
- **Cavaliere Vesely, Renata Maria Anna**
  Milano (IT)
- **Giani, Giovanni**
  Pasturago di Vernate (MI) (IT)
- **Maiocchi, Gianluigi**
  Codogno (MI) (IT)

(74) Representative: **Righetti, Giuseppe**
Bugnion S.p.A.
Viale Lancetti, 17
20158 Milano (IT)

(54) Beverages containing live lactic bacteria

(57) The invention relates to beverages for food use in combination with a mixture of lyophilized live bacteria comprising at least three bacteria species selected from *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum, Streptococcus faecium.*

EP 1 020 123 A1

Description

[0001] The present invention relates to the use of lyophilized live lactic bacteria as an addition to beverages for food use, and to a kit comprising two containers respectively holding a beverage for food use and said mixture of lyophilized live lactic bacteria intended for being added to said beverage and combined therewith at the moment of being consumed, as well as to beverages for food use containing mixtures of live lactic bacteria.

[0002] In the field of beverages, various typologies of foodstuffs are available on the market. Some of these typologies consist of beverages that are sold without any specific functional indication for consumers. This is the case of beverages that have been only studied for satisfying the consumer's thirst and therefore his/her need for drinking, and they are the so-called "ordinary beverages".

[0003] However, also available on the market are some typologies of foodstuffs consisting of beverages reproducing some "information" on their packages. The last-mentioned beverages, in addition to satisfying the consumer's thirst, also produce some beneficial effects on the organism. The composition of this kind of beverages is subjected to continuous modifications in time, depending on the commercial impulses in response to the market research and the new consumer requirements. Therefore, selection of a component to be added to a beverage, in respect of another, is not done for the purpose of characterising that type of beverage in a specific manner so as to give it those potentialities that are adapted to exert given specific functional effects on the organism. Consequently, the aim of putting on the market beverages having specific functional effects targeted at exerting particular actions on the organism has been no longer considered as a primary one.

[0004] The above described state of the art for the different beverage typologies diverges from the new food trends towards which consumers seem to be presently oriented. As a matter of fact, in the most recent years, above all in the food field, a tendency to choose foodstuffs that are functional and beneficial to the organism has become increasingly more important. That is to say, a health concept of foodstuffs has been developed by consumers. A consumer wants to eat good and healthy food, which however must be at the same time functional and active for his/her organism. Up to today in the food field of beverages no products having specific functions have been put on the market.

[0005] In fact, of all beverages available on the market no selection exists which comprises beverages having specific functions, for example beverages having either a high energy potential, a high antioxidant potential or a high multivitamin potential, in combination with lyophilized milk ferments at high concentrations to be added at the moment of consuming said beverage or previously dissolved in the beverage itself.

[0006] Creation of a selection consisting of beverages having specific functions is addressed to all consumer categories but more particularly to sportsmen that, due to their physical activity, require a special beverage having the features of a beverage with specific functions combined with the properties of a functional food.

[0007] Among functional foods, products containing a milk matrix as the major component or as an ingredient thereof and bacterial strains are known, but there are not on the market beverages that are not of a milk matrix and are just the same in combination with live lactic bacteria.

[0008] It is known that some bacteria species are considered as "probiotic", in that they perform beneficial functions for the human organism when they are present in a live and viable form in the intestinal bacterial flora.

[0009] For example some probiotic bacteria, such as the lactic bacteria specific to yoghurt (that is *Lactobacillus bulgaricus* and *Streptococcus thermophilus)* stimulate the immune system, produce antagonist effects against pathogenic microorganisms, improve lactose digestion, perform a lipolytic and proteolytic activity making fats and proteins more digestible, reduce plasmatic values of cholesterol, protect the intestinal mucosa ensuring an even assimilation of the nutritive substances, produce polysaccharides that are active on some tumors and reduce viability of some enzyme-producing microorganisms catalyzing conversion of procarcinogen substances into carcinogen substances, synthesize some important vitamins of the B group.

[0010] Other probiotic bacteria producing some of the above mentioned beneficial effects and/or contributing in a synergic manner to production of these effects and in addition producing other beneficial effects are *Bifidobacteria, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum* and *Streptococcus faecium.*

[0011] For instance, *Bifidobacteria,* in addition to stimulating the immune system, reduce the amounts of ammonia and cholesterol in the blood, promote absorption of minerals and exert a competitive exclusion of pathogenic and putrefactive bacteria. In addition, *Bilidobacteria* are deemed to exert a preventive action against the colon cancer, in that these bacteria (and more particularly *Bifidobacterium bifidum)* reduce the activity of those enzymes that convert procarcinogen substances into carcinogen substances. The last-mentioned action is performed also by *Lactobacillus acidophilus* and *Lactobacillus casei.*

[0012] Synthesis of B-group vitamins, folic acid and antioxidants, due to the action of some of the above mentioned probiotic bacteria, represents a further beneficial effect.

[0013] Only some of the above mentioned probiotic bacteria have an endogenic origin in the intestinal flora. Moreover, the intestinal bacterial flora can be reduced, become unbalanced or be eliminated not only in individuals that have been submitted to antibiotic treatments or other therapies, or suffering from inflammatory intestinal diseases, but also in apparently healthy individuals.

In addition, it is for example known that concentration of *Bifidobacteria* in the intestines is reduced with age, which will give rise to an increase in the concentration of pathogenic and putrefactive bacteria.

[0014] It is therefore important that not only probiotic bacteria that do not have an endogenic origin should be introduced into the intestinal flora, but also that the presence of the different probiotic bacteria, in a live and viable form and at high concentrations, should be ensured in the intestinal flora. It is also important that the introduced bacteria species should be suitably selected and have appropriate concentrations so that they may exert synergic and balanced actions between each other, to advantage of the host's health.

[0015] Therefore, there is still a requirement for availability on the market of beverages that do not have the drawbacks of the known art. In particular, there is still a need for a selection of beverages having specific functions capable of producing specific functional effects on the organism. That is to say, there is a need for making available on the market, functional beverages which are able to bring specific beneficial effects to the consumer's organism due to a synergy of health-nutrition effects. Functional properties are connected with the synergy between the effects caused by the specific components of the beverage in combination with selected mixtures of lyophilized live lactic bacteria.

[0016] It is an aim of the present invention to make use of beverages containing live lactic bacteria more easily accessible to, frequent and usual with any consumers, for the purpose of increasing, supplementing and balancing the intestinal flora, which will bring about advantages in terms of everyday health and sports activity.

[0017] It is a further aim of the present invention to make available on the market, functional beverages containing mixtures of live lactic bacteria, which are capable of reaching the intestines in a live and viable form, settling in the bacterial flora and growing, thereby performing important beneficial actions for the human health.

[0018] A still further aim of the present invention is to formulate mixtures of live lactic bacteria that are selected, as regards strain species and concentrations thereof, in such a manner as to perform their beneficial functions in a synergic manner with the specific type of beverage.

[0019] Another aim of the invention is to provided mixtures of live lactic bacteria to be used in combination with any beverage of a non-milk matrix, among which mineral waters, tea and others.

[0020] The foregoing and further aims that will become more apparent in the following detailed description have been reached by the Applicant's discovery according to which it has been found useful to employ selected mixtures of live lactic bacteria in combination with specific typologies of beverages.

[0021] It is therefore an object of the present invention to provide use of a mixture of lyophilized live lactic bacteria as an addition to a beverage of non-milk matrix.

[0022] It is a further object of the invention to provide a kit comprising two containers holding a non-milk matrix beverage and a mixture of lyophilized live lactic bacteria respectively, that are intended for being added to the beverage at the moment of being consumed.

[0023] Another object of the invention is to provide a beverage of non-milk matrix containing a mixture of live lactic bacteria.

[0024] The essential features of said use, kit and beverage are defined in the main claims 1, 12 and 22, respectively; some particular embodiments are defined in the dependent claims.

[0025] All percentages stated in the present specification and in claims are to be intended as percent by weight.

[0026] For preparation of the bacteria mixture, known strains of the above identified species can be used. Particularly advantageous results are achieved if the following strains are used:

- *Bifidobacterium breve:* LMG P-17501
- *Bifidobacterium infantis:* LMG P-17502
- *Bifidobacterium longum:* LMG P-17500
- *Bifidobacterium bifidum:* LMG P-17499
- *Lactobacillus acidophilus:* LMG P-17503
- *Streptococcus thermophilus:* LMG P-17225
- *Streptococcus thermophilus:* YS 46 I-1668
- *Streptococcus thermophilus:* YS 48 I-1669
- *Streptococcus thermophilus:* YS 52 I-1670
- *Lactobacillus bulgaricus:* LMG P-17224
- *Lactobacillus casei:* LMG P-17504
- *Lactobacillus plantarum:* ATCC 8014
- *Streptococcus faecium:* I - 1671
- *Streptococcus faecium:* SF 2
- *Streptococcus faecium:* SF 4

[0027] The above strains indicated by "LMG P-" are deposited with the BCCM/LMG collection of the University of Gent, Ledeganokstraat 36, B-9000 Gent, Belgium.

[0028] The above strains *Streptococcus thermophilus:* YS 46 I-1668, *Streptococcus thermophilus:* YS 48 I-1669, *Streptococcus thermophilus:* YS 52 I-1670 and *Streptococcus faecium:* I - 1671 are deposited with the CNCM - Collection Nationale de Cultures de Microorganismes - Institut Pasteur.

[0029] Strain ATCC 8014 is deposited with the American Type Culture Collection USA.

[0030] The above strains *Streptococcus faecium:* SF 2 and *Streptococcus faecium:* SF 4 are kept and commercially available with the Centro Ricerche Sitia-Yomo S.p.A.

[0031] Beverages being the object of the present invention are the result of the combination of two selections.

[0032] A first selection is addressed to determine bev-

erages having specific effects. A second selection is addressed to determine particular species of microorganisms. Combination of the two selections aims at creating a new typology of beverages called "highly functional beverages" utilizing the features of each selection in a synergic manner.

[0033] Particularly advantageous results are obtained through use of cultures at high concentrations expressed in CFU/g (where CFU means colony forming units); more particularly the following concentrations are preferred:

- *Bifidobacterium breve:* at least 30 thousand million CFU/g more preferably 50 to 70 thousand million CFU/g
- *Bifidobacterium infantis:* at least 70 thousand million CFU/g, more preferably 100 to 150 thousand million CFU/g
- *Bifidobacterium longum:* at least 30 thousand million CFU/g, more preferably 50 to 70 thousand million CFU/g
- *Bifidobacterium bifidum:* at least 50 thousand million CFU/g, more preferably 75 to 100 thousand million CFU/g
- *Lactobacillus acidophilus:* at least 50 thousand million CFU/g, more preferably 70 to 100 thousand million CFU/g
- *Streptococcus thermophilus:* at least 100 thousand million CFU/g, more preferably 150 to 200 thousand million CFU/g
- *Lactobacillus bulgaricus:* at least 5 thousand million CFU/g, more preferably 10 to 30 thousand million CFU/g
- *Lactobacillus casei:* at least 5 thousand million CFU/g, more preferably 10 to 30 thousand million CFU/g
- *Lactobacillus plantarum:* at least 100 thousand million CFU/g, more preferably 150 to 250 thousand million CFU/g
- *Streptococcus faecium:* at least 100 thousand million CFU/g, more preferably 100 to 200 thousand million CFU/g.

[0034] Cultures of the above stated concentrations are commercially available from Centro Sperimentale del Latte S.p.A. - Strada per Merlino, 3 - ZELO BUON PERSICO (Lodi)

- Italy.

[0035] Preferably in the bacteria mixtures of the present invention, the overall concentration of bacteria forming the bacteria mixture is included between 10-100 thousand million CFU/g of mixture.

[0036] The mixtures of lyophilized live lactic bacteria used in accordance with the present invention comprise at least three species of bacteria, preferably at least four species, most preferably at least five species. Said mixtures combine different species of probiotic bacteria together, utilizing all properties of same in a synergic manner. For instance, it has been found that the presence of the lactic bacteria specific to yoghurt (that is *Lactobacillus bulgaricus* and *Streptococcus thermophilus)* is particularly useful to improve viability and promote growing of other species of bacteria, such as *Bifidobacteria, Lactobacillus acidophilus* and *Lactobacillus casei.* In addition, the lactic bacteria specific to yoghurt can hydrolyze oligosaccharides that are already present in the organism, releasing sugars that are useful for enhancing growth of *Bifidobacteria* and other beneficial bacteria.

[0037] Mixtures selected for the present invention have been conceived for use in combination with any type of beverage of non-milk matrix, preferably with beverages that can be of the energy-giving, antioxidant. multivitamin types or mineral waters.

[0038] In particular, the Applicant has found that it is preferable for the mixtures of lyophilized live bacteria to be added to said beverages of non-milk matrix directly by the consumer at the moment of use.

[0039] By adding the composition at the moment of use, the consumer does not feel any soar taste, in that bacteria do not perform any fermentation activity. These bacteria are at all events viable once settled in the intestines where they develop and are active in a selective mode.

[0040] In the kit of the present invention the bacteria mixture can be preserved, if it is packaged under highly hygienic conditions, in any container protecting it from contact with air, moisture and heat, and possibly from direct light, at the temperature of a common home refrigerator (i.e. at a temperature of 4-6°C) over a period of time of 9 months and at room temperature over a period of time of 6 months.

[0041] The kit of the present invention constitutes a commercial article solving the problem of facilitating, stimulating and spreading use of highly specific functional beverages for welfare of the organism.

[0042] This type of commercial article consists of a kit comprising:

- a container X holding a beverage of non-milk matrix, and
- a container Y holding a mixture of lyophilized live lactic bacteria.

[0043] Container Y preferably contains 0.1-1 g, more preferably 0.2-0.5 g, of a mixture of lyophilized live bacteria per 500 ml of beverage contained in container X. Container Y may consist of a reservoir plug for example and container X may be selected from various shapes of practical and handy bottles that are available on the market.

[0044] Said kit must be kept at the temperature at which the composition of container Y must be maintained and can be preserved over periods of time as long

as six months at room temperature and over periods of time as long as nine months at the temperature of a refrigerator, i.e. included between 4 and 6°C. Once it has been bought, the bottle containing the beverage with the reservoir plug holding the mixture of lactic bacteria annexed thereto can be preserved in a common home refrigerator or at room temperature and can be used at any moment by opening both containers (bottle and reservoir plug) and mixing contents thereof at the moment the beverage is wished to be drunk.

[0045] Particular preferred embodiments of the present invention consist in use of mixtures of lyophilized live lactic bacteria as an addition to an energy-giving beverage, an antioxidant beverage, a multivitamin beverage, and mineral water.

[0046] An energy-giving beverage in accordance with the present invention preferably comprises:

- water, in an amount preferably included between 70 and 90%;
- fruit juice, preferably orange juice, in an amount preferably included between 5 and 12%;
- vitamins, preferably vitamin B1, B2, B6, niacin, to such an amount that at least 30% of the recommended daily allowance (RDA) is supplied, with reference to energy-giving beverage volumes of 500 ml;
- creatine or carnitine in amounts preferably included between 2 and 3 g/500 ml of energy-giving beverage and 0.1 and 0.2 g/ml of energy-giving beverage, respectively;
- mineral salts, preferably Mg and K salts, in amounts included between 30 and 40 mg/500 ml of energy-giving beverage and 55 and 65 mg/500 ml of energy-giving beverage, respectively;
- sugars, preferably sucrose and/or fructose and/or maltodextrins in a total amount included between 7 and 12%;
- optional flavors in a total amount included between 0.05 and 0.2%;
- a mixture of lyophilized lactic bacteria preferably including the following strains:
- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, in a total amount preferably included between 45 and 55%;
- *Lactobacillus acidophilus*, in an amount preferably included between 30 and 40%;
- *Lactobacillus casei*, in an amount preferably included between 5 and 10%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum in an amount preferably included between 5 and 10%, of which *Bifidobacterium bifidum* in an amount included between 65 and 85%.

[0047] The Applicant has found that said mixture of lyophilized lactic bacteria made up of particular carefully selected strains of bacteria and in right proportions performs an important functional role on the organism. The functional character is due to the fact that this specific mixture of bacteria takes part in:

- keeping a water/solid balance in the intestinal bolus;
- carrying out the hydrolysis of polysaccharides into simpler energy-giving compounds such as trisaccharides and tetrasaccharides that are therefore immediately available for the host;
- keeping the mucosa permeability stable, so as to ensure transportation of the nutrients through the intestinal membrane;
- forming vitamin complexes of the B group, with particular reference to vitamin B1, B2, B12 and to folic acid that will further implement the amount carried through the beverage;
- forming short-chain fat acids such as acetic acid, butyric acid, propionic acid that represent simple energy-giving elements available for the host;
- forming peptides and even essential amino acids which are available as plastic and energy-giving source for the host;
- reducing the intestinal pH with modification of the acid-base balance, thereby promoting absorption of mineral substances (greater bioavailability);
- inhibiting deamination and microbial decarboxylation of amino acids while at the same time ensuring saving in food amino acids; and
- performing hepato-protecting functions by deconjugating bile acids.

[0048] The Applicant has found that the functional quality carried out by said mixture of lactic bacteria in a synergic manner with the functional quality carried out by the other beverage components ensures a high and quick energy saving for the consumer host.

[0049] In putting the invention into practice in the form of a kit, the lyophilized bacteria mixture is preferably contained in a reservoir plug and the overall concentration of lyophilized lactic bacteria is preferably included between 10 and 100 thousand millions per gram of bacteria mixture. The ferment amount added to the energy-giving beverage is preferably included between 0.1 and 1 g per 100 ml of beverage; consequently the milk ferment content after dissolution in the beverage, preferably at the moment of drinking the same, is included between 1 and 100 thousand millions per 500 ml of beverage. The energy-giving beverage has a caloric contribution of about 210 Kcal/500 ml.

[0050] Another preferred embodiment being the object of the present invention consists of an antioxidant green-tea beverage preferably comprising:

- water, in an amount preferably included between 72 and 92%;
- green-tea extract, in an amount preferably included

between 0.1 and 0.25%;
- tea extract, in an amount preferably included between 0.01 and 0.2%;
- fruit juice, preferably lemon juice, in an amount included between 1 and 5%;
- antioxidant vitamins, preferably of the group A, C, E, to such an amount that at least 30% of the recommended daily allowance (RDA) is supplied per 500 ml of beverage;
- selenium, preferably in a total amount included between 6 and 10 mg;
- sugars, preferably sucrose in a total amount included between 6 and 10%;
- optional flavors in a total amount included between 0.05 and 0.2%;
- a mixture of lyophilized lactic bacteria preferably including the following strains:
- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, in a total amount preferably included between 50 and 60%,
- *Lactobacillus acidophilus*, in an amount preferably included between 35 and 45%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum in an amount preferably included between 5 and 15%, of which *Bifidobacterium bifidum* in an amount included between 75 and 85%.

- *Streptococcus faecium*, in an amount preferably included between 5 and 10%.

[0051] The Applicant has found that the above mixture of lyophilized lactic bacteria made up of particular bacteria strains which have been carefully selected and are in the right proportions, performs an important functional role on the organism. The functional character is due to the fact that this specific mixture of bacteria takes part in:

- reducing the flora that produces procarcinogen enzymes such as nitroreductase and β-glucuronidase by preventing formation of nitrosamines thereby completing the antioxidant action of vitamin C;
- deconjugating the primary bile acids, thereby exerting a hepato-protecting action;
- regulating the immune-competence from a local and systemic point of view; and
- carrying out a detoxicating action through reduction of the urease-positive flora or neutralization of toxins.

[0052] The Applicant has found that by the above mentioned functions performed by the bacteria mixture set forth above a synergic action is obtained which is complementary to the specifically antioxidant functions carried out by the beverage components.

[0053] In putting the present invention into practice in the form of a kit the lyophilized bacteria mixture is preferably contained in a reservoir plug and the overall concentration of the lyophilized lactic bacteria is preferably included between 10 and 100 thousand millions per gram of the bacteria mixture. The amount of ferments added to the antioxidant beverage is preferably included between 0.1 and 1 g per 500 ml of beverage; consequently the milk ferment content after dissolution in the beverage, preferably at the moment of drinking the same, is included between 1 and 100 thousand millions per 500 ml of beverage. The antioxidant beverage has a caloric contribution of about 200 Kcal/500 ml.

[0054] Another preferred embodiment being the object of the present invention consists of a multivitamin beverage preferably comprising:

- water, in an amount preferably included between 55 and 85%;
- fruit juice, preferably orange and/or carrot and/or lemon juice, in an amount preferably included between 10 and 25%, 4 and 8% and 1 and 5%, respectively;
- vitamins, preferably of the C group, niacin, provitamin A (β-Carotene), E, pantothenic acid, B6, B2, folacin, biotin, B12 to such an amount that 15-30% of the recommended daily allowance (RDA) is ensured per 500 ml of beverage;
- sugars, preferably sucrose in a total amount included between 4 and 7%;
- optional flavors in a total amount included between 0.05 and 0.2%;
- a mixture of lyophilized lactic bacteria preferably including the following strains:
- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, in a total amount preferably included between 20 and 30%;
- *Lactobacillus acidophilus*, in an amount preferably included between 40 and 60%;
- *Lactobacillus casei*, in an amount preferably included between 20 and 40%;
- *Lactobacillus plantarum*, in an amount preferably included between 5 and 10%.

[0055] The Applicant has found that said mixture of lyophilized lactic bacteria made up of particular bacteria strains which have been carefully selected and are in right proportions, performs and important functional role on the organism, which in a synergic manner supplements the functional role performed by the other beverage components.

[0056] The functional character is due to the fact that this specific bacteria mixture takes part in:

- maintaining the autochthonous intestinal flora balance enabling a regular synthesis of the vitamins of the B group and vitamins K;
- stabilizing the permeability of the cell membrane of

the gastrointestinal system, enabling absorption of the diet vitamins;
- cooperating with vitamin E of the diet in maintaining the permeability features of the membrane; and
- carrying out synthesis of the vitamins, referring particularly to B, PP, K complex, folic acid and pantothenic acid.

[0057] In putting the present invention into practice in the form of a kit, the lyophilized bacteria mixture is preferably contained in a reservoir plug and the overall concentration of the lyophilized lactic bacteria is preferably included between 10 and 100 thousand millions per gram of bacteria mixture. The amount of ferments added to the multivitamin beverage is preferably included between 0.1 and 1 g per 500 ml of beverage; consequently the milk ferment content after dissolution in the beverage, preferably at the moment of drinking the same, is included between 1 and 100 thousand millions per 500 ml of beverage. The multivitamin beverage has a caloric contribution of about 190 Kcal/500 ml.

[0058] In addition, the Applicant has found it useful to conceive fizzy and natural mineral water beverages providing addition of lactic bacteria mixtures, preferably in a proportion of 0.1-1 g/500 ml of mineral water.

[0059] Preferentially, the involved mineral water is a natural mineral water low in mineral content, which contains the following ions:

- sodium ion, at a concentration of about 7.1 mg/l;
- potassium ion, at a concentration of about 1.2 mg/l;
- magnesium ion, at a concentration of about 27 mg/l;
- calcium ion, at a concentration of about 46 mg/l;
- hydrocarbon ion, at a concentration of about 275 mg/l;
- hydrochloric ion, at a concentration of about 2.4 mg/l;
- sulphuric ion, at a concentration of about 6.3 mg/l;
- silica (expressed as SiO_2), at a concentration of about 16 mg/l;
- traces of hydrofluoric ion and strontium.

[0060] Preferably the bacteria mixture to be added to mineral water has the following composition:

- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, in a total amount preferably included between 40 and 60%;
- *Lactobacillus acidophilus*, in an amount preferably included between 5 and 15%;
- *Lactobacillus casei*, in an amount preferably included between 4 and 8%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* in an amount preferably included between 20 and 30%, of which *Bifidobacterium bifidum* in an amount included between 60 and 80%;

- *Streptococcus faecium*, in an amount preferably included between 2 and 7%;
- *Lactobacillus plantarum*, in an amount preferably included between 3 and 8%.

[0061] From the above it appears that use of mixtures of lyophilized bacteria in combination with any beverage by a kit (consisting of a small bottle and a reservoir plug, for example) being the object of the present invention is simple, flexible and very useful because it promotes welfare of the human organism by detoxicating it as well as normalizing and boosting the intestinal flora functions.

[0062] The following examples are reproduced for the purpose of illustrating some embodiments of the invention, without however limiting the range thereof.

[0063] The bacteria concentrations used in the below examples are the following:

- *Bifidobacterium breve:* 50 thousand million CFU/g;
- *Bifidobacterium infantis:* 110 thousand million CFU/g;
- *Bifidobacterium longum:* 55 thousand million CFU/g;
- *Bifidobacterium bifidum:* 80 thousand million CFU/g;
- *Lactobacillus acidophilus:* 90 thousand million CFU/g;
- *Streptococcus thermophilus:* 170 thousand million CFU/g;
- *Streptococcus thermophilus:* YS 46 I-1668
- *Lactobacillus bulgaricus:* 15 thousand million CFU/g;
- *Lactobacillus casei:* 12 thousand million CFU/g;
- *Lactobacillus plantarum:* 160 thousand million CFU/g; and
- *Streptococcus faecium:* 150 thousand million CFU/g.

Example 1 (energy-giving beverage)

[0064] The bacteria mixture/beverage proportion is 0.2 g of bacteria mixture per 500 ml of beverage. The bacteria mixture composition is the following:

- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, at a concentration of 50%
- *Lactobacillus acidophilus*, at a concentration of 40%;
- *Lactobacillus casei*, at a concentration of 5%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* at a concentration of 5%, of which *Bifidobacterium bifidum* at a concentration of 70%.

[0065] The beverage composition is the following:

- water 80%,
- orange juice 10%;
- vitamins B1, B2, B6 and niacin 8 mg/500 ml;
- creatine 1.9 mg/500 ml;
- mineral salts Mg and K 30 mg/500 ml;
- sugars: sucrose 4%; fructose 3% and maltodextrins 3%; and
- flavors 0.1%.

Example 2 (antioxidant beverage)

[0066] The bacteria mixture/beverage proportion is 0.3 g of bacteria mixture per 500 ml of beverage.

[0067] The bacteria mixture composition is the following:

- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, at an overall concentration of 50;
- *Lactobacillus acidophilus*, at a concentration of 36%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum at a concentration of 6%, of which *Bifidobacterium bifidum* at a concentration of 80%.

- *Streptococcus faecium*, at a concentration of 8%.

[0068] The beverage composition is the following:

- water 90%;
- green tea extract 0.1%;
- tea extract 0.1%;
- lemon juice 2%;
- vitamins: A, C and E 60 mg/500 ml;
- selenium 8 mg/500 ml;
- sugars 7.7%, and
- flavors 0.1%.

Example 3 (multivitamin beverage)

[0069] The bacteria mixture/beverage proportion is 0.1 g of bacteria mixture per 500 ml of mineral water.

[0070] The bacteria mixture composition is the following:

- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, at an overall concentration of 20;
- *Lactobacillus acidophilus*, at a concentration of 50%;
- *Lactobacillus casei*, at a concentration of 20%;
- *Lactobacillus plantarum*, at a concentration of 10%.

[0071] The composition of the mineral water is the following:

- water 70%
- orange juice 15%;
- carrot juice 5%;
- lemon juice 4%;
- vitamins: C, niacin, provitamin A, E, pantothenic acid, B6, B2, folacin, biotin, and B12 at an overall concentration of 75 mg/500 ml;
- sugars 5.85%; and
- flavors 0.15%.

Example 4 (natural low-in-mineral-content water)

[0072] The bacteria mixture/beverage proportion is 0.3 g of bacteria mixture per 500 ml of mineral water.

[0073] The bacteria mixture composition is the following:

- *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, at an overall concentration of 50%;
- *Lactobacillus acidophilus*, at a concentration of 10%;
- *Lactobacillus casei*, at a concentration of 5%;
- A mixture of *Bifidobacteria* consisting of:

Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum at an overall concentration of 25%, of which *Bifidobacterium bifidum* at a concentration of 75%;

- *Streptococcus faecium*, at a concentration of 5%;
- *Lactobacillus plantarum*, at a concentration of 5%.

[0074] The mineral water composition is the following:

- sodium ion, at a concentration of 7.2 mg/l;
- potassium ion, at a concentration of 1.1 mg/l;
- magnesium ion, at a concentration of 26 mg/l;
- calcium ion, at a concentration of 47 mg/l;
- hydrocarbon ion, at a concentration of 274 mg/l;
- hydrochloric ion, at a concentration of 2.3 mg/l;
- sulphuric ion, at a concentration of 6.2 mg/l;
- silica (expressed as SiO_2), at a concentration of 17 mg/l;
- traces of hydrofluoric ion and strontium.

Claims

1. Use of a mixture of lyophilized live lactic bacteria comprising at least three bacteria species selected from *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum* and *Streptococcus faecium* as an addition to a beverage of non-milk matrix.

2. Use as claimed in claim 1, wherein the lactic bacte-

ria mixture comprises at least four species.

3. Use as claimed in claim 1 and/or 2, wherein the overall concentration of bacteria forming the mixture is included between 10 and 100 thousand million CFU/g of bacteria mixture.

4. Use as claimed in one or more of the preceding claims, wherein the concentration of bacteria used for preparing the bacteria mixture are the following:

- *Bifidobacterium breve:* at least 30 thousand million CFU/g;
- *Bifidobacterium infantis:* at least 70 thousand million CFU/g;
- *Bifidobacterium longum:* at least 30 thousand million CFU/g;
- *Bifidobacterium bifidum:* at least 50 thousand million CFU/g;
- *Lactobacillus acidophilus:* at least 50 thousand million CFU/g;
- *Streptococcus thermophilus:* at least 100 thousand million CFU/g;
- *Lactobacillus bulgaricus:* at least 5 thousand million CFU/g;
- *Lactobacillus casei:* at least 5 thousand million CFU/g;
- *Lactobacillus plantarum:* at least 100 thousand million CFU/g; and
- *Streptococcus faecium:* at least 100 thousand million CFU/g.

5. Use as claimed in one or more of the preceding claims, wherein addition of the bacteria mixture is carried out in a proportion of 0.1 - 1 g per 100 ml of beverage of non-milk matrix.

6. Use as claimed in one or more of the preceding claims, wherein addition of the bacteria mixture to the beverage is carried out at the moment of drinking the same.

7. Use as claimed in one or more of the preceding claims, wherein the beverage is mineral water, or a beverage comprising water and at least three other components selected from fruit juices, carrot juice, vitamins, creatine, carnitine, mineral salts, selenium, tea extract, green tea extract, sugars and flavors.

8. Use as claimed in one or more of the preceding claims, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum* and *Bifidobacterium bifidum* and the beverage has a composition comprising: water, fruit juice, vitamins, creatine and/or carnitine, mineral salts and sugars.

9. Use as claimed in one or more of claims 1 to 7, wherein the bacteria species are selected from *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus faecium* and the beverage has a composition comprising: water, green tea extract, tea extract, fruit juice, vitamins, antioxidants, selenium and sugars.

10. Use as claimed in one or more of claims 1 to 7, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum* and the beverage has a composition comprising: water, fruit and/or carrot juice, vitamins and sugars.

11. Use as claimed in one or more of claims 1 to 7, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus faecium, Lactobacillus plantarum,* and the beverage is mineral water.

12. A kit comprising:

- a container X holding a beverage of non-milk matrix, and
- a container Y holding a mixture of lyophilized live lactic bacteria comprising at least three bacteria species selected from *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum and Streptococcus faecium,* both said containers being closed and intended for opening at the moment of use of said non-milk matrix beverage, and said container Y being intended for the purpose of adding the mixture of lyophilized live lactic bacteria therein contained to said beverage at the moment of drinking the same.

13. A kit as claimed in claim 12, wherein the lactic bacteria mixture comprises at least four species.

14. A kit as claimed in claim 12 and/or 13, wherein the overall concentration of bacteria forming the mixture is included between 10 and 100 thousand million CFU/g of bacteria mixture.

15. A kit as claimed in one or more of claims 12 to 14,

wherein the concentration of bacteria used for preparing the bacteria mixture are the following:

- *Bifidobacterium breve:* at least 30 thousand million CFU/g;
- *Bifidobacterium infantis:* at least 70 thousand million CFU/g;
- *Bifidobacterium longum:* at least 30 thousand million CFU/g;
- *Bifidobacterium bifidum:* at least 50 thousand million CFU/g;
- *Lactobacillus acidophilus:* at least 50 thousand million CFU/g;
- *Streptococcus thermophilus:* at least 100 thousand million CFU/g;
- *Lactobacillus bulgaricus:* at least 5 thousand million CFU/g;
- *Lactobacillus casei:* at least 5 thousand million CFU/g;
- *Lactobacillus plantarum:* at least 100 thousand million CFU/g;
- *Streptococcus faecium:* at least 100 thousand million CFU/g.

16. A kit as claimed in one or more of claims 12 to 15, wherein container Y hold 0.1-1 g of a mixture of lyophilized live bacteria per 500 ml of non-milk matrix beverage of container X.

17. A kit as claimed in one or more of claims 12 to 16, wherein the beverage is mineral water or a beverage comprising water and at least three other components selected from fruit juices, carrot juice, vitamins, creatine, carnitine, mineral salts, selenium, green tea extract, sugars and natural flavors.

18. A kit as claimed in one or more of claims 12 to 17, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum* and *Bifidobacterium bifidum* and the beverage has a composition comprising: water, fruit juice, vitamins, creatine and/or carnitine, mineral salts and sugars.

19. A kit as claimed in one or more of claims 12 to 17, wherein the bacteria species are selected from *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum* and *Streptococcus faecium* and the beverage has a composition comprising: water, green tea extract, tea extract, fruit juice, vitamins, antioxidants, selenium and sugars.

20. A kit as claimed in one or more of claims 12 to 17, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum* and the beverage has a composition comprising: water, fruit and/or carrot juice, vitamins and sugars.

21. A kit as claimed in one or more of claims 12 to 17, wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus faecium, Lactobacillus plantarum,* and the beverage is mineral water.

22. A beverage of non-milk matrix containing a mixture of live lactic bacteria comprising at least three bacteria species selected from the following: *Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum and Streptococcus faecium.*

23. A beverage as claimed in claim 22 wherein the bacteria mixture comprises at least four species.

24. A beverage as claimed in claim 22 and/or 23, wherein the overall concentration of bacteria forming the mixture is included between 10 and 100 thousand million CFU/g of bacteria mixture.

25. A beverage as claimed in one or more of claims 22 to 24, wherein the concentration of bacteria used for preparing the bacteria mixture are the following:

- *Bifidobacterium breve:* at least 30 thousand million CFU/g;
- *Bifidobacterium infantis:* at least 70 thousand million CFU/g;
- *Bifidobacterium longum:* at least 30 thousand million CFU/g;
- *Bifidobacterium bifidum:* at least 50 thousand million CFU/g;
- *Lactobacillus acidophilus:* at least 50 thousand million CFU/g;
- *Streptococcus thermophilus:* at least 100 thousand million CFU/g;
- *Lactobacillus bulgaricus:* at least 5 thousand million CFU/g;
- *Lactobacillus casei:* at least 5 thousand million CFU/g;
- *Lactobacillus plantarum:* at least 100 thousand million CFU/g; and
- *Streptococcus faecium:* at least 100 thousand million CFU/g.

26. A beverage as claimed in one or more of claims 22 to 25, wherein the lactic bacteria mixture is present in a proportion of 0.1-1 g per 500 ml of a non-milk matrix beverage.

27. A beverage as claimed in one or more of claims 24 to 26, wherein said beverage is mineral water or a beverage comprising water and at least three other components selected from fruit juices, carrot juice, vitamins, creatine, carnitine, mineral salts, selenium, tea extract, green tea extract, sugars and natural flavors.

28. A beverage as claimed in one or more of claims 22 to 27, wherein said beverage has a composition comprising water, fruit or carrot juice, vitamins, creatine and/or carnitine, mineral salts and sugars, and wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum* and *Bifidobacterium bifidum.*

29. A beverage as claimed in one or more of claims 22 to 27, wherein said beverage has a composition comprising water, green tea extract, tea extract, fruit juice, vitamins, antioxidants, selenium and sugars and wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus faecium.*

30. A beverage as claimed in one or more of claims 22 to 27, wherein said beverage has a composition comprising water, fruit and/or carrot juice, vitamins and sugars and wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum.*

31. A beverage as claimed in one or more of claims 22 to 27, wherein said beverage is mineral water and wherein the bacteria species are selected from: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium bifidum, Streptococcus faecium, Lactobacillus plantarum.*

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 83 0013

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	5 July 1999	Van Moer, A

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X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

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EXHIBIT D

US006156355A

United States Patent [19]

Shields, Jr. et al.

[11] Patent Number: 6,156,355

[45] Date of Patent: Dec. 5, 2000

[54] **BREED-SPECIFIC CANINE FOOD FORMULATIONS**

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[51] **Int. Cl.**[7] **A23K 1/175**

[52] **U.S. Cl.** **426/74**; 426/61; 426/805; 426/650

[58] **Field of Search** 426/74, 61, 805, 426/650

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Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis, L.L.P.

[57] **ABSTRACT**

Breed-specific dog food formulations that comprise chicken meat as the major ingredient, rice as the predominant (or sole) grain source, fruit and/or vegetable fiber as the primary or sole fiber source, unique fat and antioxidant blend, vitamins, herbs and spices, carotenoids, and no corn or artificial colors, preservatives, flavors or sugars are provided.

7 Claims, No Drawings

BREED-SPECIFIC CANINE FOOD FORMULATIONS

RELATED APPLICATIONS

This application claims benefit of priority to provisional application Serial No. 60/107,033, filed Nov. 2, 1998, the contents of which are incorporated by reference in their entirety herein.

FIELD OF THE INVENTION

This invention is related to pet food formulations designed based on the unique characteristics of different breeds.

BACKGROUND OF THE INVENTION

A wide variety of different dog food formulations of the wet and dry type are commercially available, e.g., from grocery, pet specialty and veterinary sources. Typically, such dog food formulations are generic in that they are designed to be consumed by any breed. However, some dog food formulations are specialized at least to the extent that they are preferably consumed by dogs of different ages, e.g, puppy, adult and geriatric dog formulations are widely available. Typically, such formulations differ in their caloric, protein, and fat content. Also, dog food formulations adapted for obese dogs are prevalent in the industry. Such dog food formulations, as might be expected, typically have a lower caloric content and higher fiber content relative to other dog food formulations.

In fact, there are many excellent premium pet foods in the marketplace which address the nutritional needs of pets from different perspectives. A good example of pet food formulations that address nutritional needs based on specific age are the Nature's Recipe Original® products. These diets address specific nutrient needs during an individual life stage. These diet lines differ widely with respect to the true differences among life stages. Because of the wide differences in rate at which breeds mature and absolute life-span differences, determination of the appropriate time to switch diets can be quite difficult. Other products address different energy requirements among pets, some of which may have a genetic component. The Puppy/Performance/-Lactation, Maintenance and Senior/Pension Lamb Meal & Rice Formulations incorporate a range of dietary energy levels as wide as any in the industry.

An additional segment of the pet food market incorporates differences in ingredient usage or product form. These fill the needs of some pet owners for taste and variety. Nature's Recipe® diets are well-represented in this segment as well since diets are formulated using a single meat protein source, allowing for true ingredient diversity. Many other product lines are fairly similar in ingredient selection with only minor differences in formulations. Relative to product form, Nature's Recipe Original® includes a complementary line of dry and canned products. Additionally, within canned products they offer a choice of ground or formed (carved) products.

A final segment of the pet food market which has been recently introduced involves formulations for specific breeds and/or adult weight. The Nature's Recipe® Group Specific Formulas excel in this category as they consider differences in nutrient requirements and physical form (size and mouth configuration) among breeds.

Such formulations have been developed in part because canine breeds differ from each other both on the outside and the inside. These differences include some of the more obvious things, including size at maturity, mouth and body dimensions. They also differ with respect to how fast they reach their mature weight. Some breeds, such as the Miniature Dachshund, reach mature body weight at approximately eight months of age while the Newfoundland takes over two years. This makes a huge difference in the physiological maturity of the dam at breeding age.

Although not marked, differences do exist in the digestibility of nutrients among breeds. While no comprehensive research for all breeds have been done, some research conducted by the present inventors has suggested that Toy Fox Terriers had a lower digestibility coefficient for the same diet than for the Beagle or Brittany, and that daily energy requirements to maintain body weight are lower for Miniature Poodles and higher for German Shorthairs and Coonhounds than the other breeds at our kennel. The latter likely resulted from the activity level of these breeds as they were quite active. On the same note, some breeds, such as Labrador Retrievers, Basset Hounds, Beagles, Cocker Spaniels, and several in the Terrier group, appear to be predisposed to obesity.

Finally, the ingredient tolerance and nutrient metabolism differ among breeds. This may result in different "normal" blood measurements. Published research has documented that, at least during the reproductive cycle, the Brittany has inherently lower levels of some standard blood measurements than the Beagle or Labrador Retriever. Some of these differences result from efficiencies of various enzyme systems in the body while others are the result of actual genetic abnormalities which accumulate over generations of breeding.

There have been 350–400 genetic disorders identified in dogs, compared to approximately 3,200 in humans. These disorders have been identified in approximately seventy percent of the recognized breeds and the prevalence in all dogs is thought to be approximately twenty-five percent. These genetic difficulties include anatomical malformations, errors of metabolism and genetic predispositions to conditions including cancer, bleeding disorders, and drug reaction. Often times the problem is an enzyme deficiency or defect in a specific structural protein in the body. This, in turn, results in a deficiency of some compound required by the body, a build-up of a compound at unusually high levels, or adaptation of metabolic pathways in the body to compensate for the problem. In the latter situation, clinical signs may not be evidenced or may only manifest themselves at times of high nutrient need. Some genetic defects are lethal either to the developing fetus or early in life, while others are not life-threatening.

Many of these conditions are inherited recessively and do not show up until two dogs which are carriers are bred. Unfortunately, screening tests are not available for many conditions and breeders often find out about problems only after a stud dog has been bred to numerous females. A carrier with an excellent show record can, therefore, spread the gene rapidly. It has been estimated that a single stud dog could represent five to ten percent of the entire genetic make-up of some rare breeds. Inbreeding per se is not necessarily the cause of expression of genetic problems but does expose them more readily. All biological organisms accumulate mutations over time and those which have fatal consequences become self limiting. Moreover, genetic defects are not exclusive to purebreds. It has been estimated that mixed breed dogs have 102 known defects, which is greater than many purebred breeds with a high incidence such as Cairn Terriers (37) and Cocker Spaniels (52). The reason for this is that many breeds have common genetic defects.

As indicated above, many healthy humans have genetic defects which force them to consume special diets. Any person who drinks a diet cola will find the phrase "phenylketonurics: contains phenylalanine" because some people have an inability to metabolize this amino acid (protein building block) so they should try to limit consumption. Another well known condition is lactose intolerance (inability to digest and utilize milk sugar) present in humans, especially those of Asian, Southern European, or African decent. The lack of persistence of the ability to digest milk after weaning is carried as a recessive gene. This situation is easily handled by avoidance of milk or consumption of enzymes which assist this digestion. A minority of the population in unable to regulate cholesterol synthesis in response to dietary intake, but it seems to be a dietary consideration in all of us. Incidence of most of the chronic diseases which occur in the geriatric population, including degenerative joint disease, heart disease, liver disease and diabetes also likely have genetic components.

Thus, pets, similar to humans, exhibit significant genetic diversity which affects their overall health and nutritional requirements. Therefore, notwithstanding the many different types of pet food formulations, and more specifically dog food formulations commercially available, there still exists a prevalent need for improved formulations that take into account the significant genetic differences between different breeds.

OBJECTS OF THE INVENTION

Accordingly, it is an object of the invention to provide improved dog food formulations designed for specific breeds that are designed based on the genetic diversity of different dog breeds.

More specifically, it is an object of the invention to provide dog food formulations that are designed taking into account the different food allergies of different dog breeds.

Still more specifically, it is an object of the invention to provide dog food formulations that comprise the following unique combination of ingredients and features:

(i) chicken meat and/or meal as the primary ingredient (and only meat source);
(ii) rice as the primary grain source;
(iii) unique antioxidant blend;
(iv) unique fat blend
(v) organic minerals;
(vi) unique fiber blend;
(vii) specific combinations of herbs and species;
(viii) no added artificial colors or preservatives, flavors or sugars; and
(ix) nutrition substantiation through AAFCO feeding studies.

More specifically, it is an object of the invention to provide pet food formulations having the above ingredients and features wherein:

(1) the total digestibility ranges from 85–90%;
(2) there are no meat products other than chicken meat and/or meal;
(3) it lacks any corn;
(4) it comprises a blend of vitamins including tocopherols, vitamin C (ascorbic acid), minerals (copper, zinc and iron in inorganic and organic complex form), carotenoids (e.g., beta carotene and lutein), and herbs (including rosemary);
(5) a fat blend including canola oil, salmon oil and evening primrose oil;
(6) fruit and/or vegetable fiber rather than grains, such as tomato pomace, as the primary fiber source.
(7) herbs and spices including spearmint, ginger, ginseng, ginkgo, parsley and *Yucca schidigera* extract; and
(8) kibble size, shape, feed recommendations tailored to specific breed.

Still more specifically, it is an object of the invention to provide a dog food formulation specially designed for sporting dogs. In particular, formulations designed for sporting dogs will comprise rice, no gluten-containing grains, taurine, Vitamin E, selenium, and herbs, and are fortified with sodium bicarbonate and minerals, such as calcium, and organic compounds such as glucosamine. Also, this formulation has higher percentage of fat calories and energy relative to other breed formulations. This diet is made in a unique triangle shape which resembles the mouth dimensions of breeds in this group.

It is another more specific object of the invention to provide a dog food formulation that comprises the foregoing ingredients and features which is specifically designed for working dogs that contains a high percentage of fat, higher vitamin and mineral fortification, and which is fortified with a number of antioxidants, choline, garlic, Hawthorn berry powder, and taurine, and minerals including calcium, and organic compounds such as glucosamine, potassium citrate, and sodium.

It is still another specific object of the invention to provide dog food formulations that comprises the foregoing general ingredients and features that are specifically adapted for non-sporting dogs. Such formulations will comprise, in particular, sodium hexametaphosphate, and significant amounts of Vitamin A, B Vitamins, and minerals such as copper and zinc.

It is yet another object of the invention to provide a dog food formulations that comprise the foregoing general ingredients and features that are specifically designed for herding dogs. The herding dog formulations comprise higher vitamin and mineral supplements, such as calcium, potassium citrate, and sodium, and organic compounds such as glucosamine, and additionally comprise oat and barley fiber, direct-fed microbials (DFM), and bromelain (to aid digestion).

It is still another object of the invention to provide dog food formulations that comprise the foregoing general ingredients and features which are specifically designed for Terrier dogs. These formulations comprise higher protein and immediate fat content, lower copper and higher zinc, garlic and milk thistle powder.

It is another specific object of the invention to provide dog food formulations comprising the above features and ingredients that are designed for Toy breeds. These formulations further comprise sodium bicarbonate and potassium chloride, garlic powder, cranberry powder, niacin, B-vitamins, yeast, and have a small kibble size because of the small size of Toy breeds.

It is another object of the invention to provide dog food formulations which additionally comprise the foregoing general ingredients and features that are specifically designed for hounds. These formulations comprise higher vitamin and mineral levels, glutamine, oat and barley fiber, direct-fed microbials (DFM), garlic and cranberry juice powder.

DETAILED DESCRIPTION OF THE INVENTION

The breed-specific dog formulations of this invention were originally developed because of a recognized food

sensitivity observed in different types of dogs which undoubtedly had a genetic component. Numerous well-recognized problems exist in individual breeds, including a Vitamin A responsive dermatitis in Cocker Spaniels and zinc-responsive dermatitis in Siberian Huskies and Alaskan Malamutes. An additional example is the presence of a gluten intolerance in Irish Setters which closely resembles celiac disease in humans and manifests itself as weight loss and chronic, intermittent diarrhea. German Shepherds and Beagles, on the other hand, appear to experience diarrhea caused by a gastrointestinal immune deficiency. Hip dysplasia has been identified in over 100 breeds including several in the Herding, Working and Sporting group. The cause of this condition is likely multifactorial in nature, but dietary management may play a role in expression of the condition. Some Cocker Spaniels and Golden Retrievers appear to have low blood taurine levels which are responsive to dietary taurine supplementation, similar to the cat. Dalmatians are recognized as having predisposition to deafness and presence of uric acid crystals in the urine. Several breeds within the Working Group, including Boxers, Doberman Pinschers, and Great Danes, can develop a heart condition called cardiomyopathy. Bedlington Terriers and West Highland Terriers can experience a copper storage disease. Poodles have been recognized as having somewhat of a predisposition toward periodontal disease.

The pet food formulations of this invention, which are commercially available under the tradename Nature's Recipe Group Specific Formulas® are designed to meet the unique needs of pets within various breed groups. They share many common characteristics which make them excellent choices for any pet and some unique characteristics which may add additional value for specific breed groups. Common Features of Feed Formulations of the Invention

The novel breed-specific dog food formulations of the invention now being sold under the name Nature's Recipe Group Specific Formulas® contain many common features which collectively make them unique both from previously available Nature's Recipe® products and other pet food formulations. These include:

- Chicken meat as the number one ingredient—15–30%, preferred 20–25%.
- Rice as the primary grain source—20 to 45%.
- Unique antioxidant blend—Tocopherols (0.025 to 0.05%), Vitamin C, beta carotene, lutein (from marigold extract), lycoper (from tomato pomace), and rosemary, ginkgo and ginseng
- Organic minerals—0.1 to 0.2%
- Unique fiber blend—0.5 to 4%
- Herbs and Spices—0.05 to 0.2%
- No added artificial colors, preservatives, flavors or sugars
- Nutrition substantiation through AAFCO feeding studies.

The combination of chicken meat, quality grains, such as rice, and herbs and spices, assures unique taste and nutrition. Total digestibility of these formulations ranges from 85–90%, well above those of competitive pet food products. Preferably, the crude protein of the subject formulations will range from about 20 to 30% minimum, more preferably about 22 to 26% minimum.

Free radicals which form upon exposure to the environment or during normal metabolism can be harmful to cell membranes, proteins and genetic material which can have adverse consequences on the quality of the food and to the body as well. Accordingly, the subject formulations comprise a balanced blend of antioxidants with respect to solubility (fat or water soluble), stage of rancidity in which they act (oxygen scavengers, free radical termination), and tissues in which they concentrate in the body. Additionally, some antioxidants are complementary and others antagonistic to one another, so balance of these antioxidants is crucial. Accordingly, the subject formulations have been designed to incorporate a blend of vitamins (tocopherols, Vitamin C (ascorbic acid), minerals (copper, zinc and iron in both inorganic and organic complex form), carotenoids (such as beta carotene and lutein from marigold extract) and herbs (including rosemary), to perform this very important function.

Moreover, the formulations of the invention comprise a unique fat blend which includes canola oil, salmon oil, and evening primrose oil to complement the excellent fat quality of the chicken fat. Canola oil and salmon oil are used as sources of short and long-chain omega-3 fatty acids, while evening primrose oil provides an omega-6 fatty acid called gamma linolenic acid (GLA). The latter two fat sources bypass the need for some key enzymes required in fatty acid metabolism. The present inventors hypothesize that both the ratio of omega-6 to omega-3 fatty acids and the absolute quantities of individual representative compounds is significant to the nutritional requirements of dogs.

Preferably, the minimum amount of canola oil will range from 1 to 5.0 percent, the minimum amount of salmon oil from 0.1 to 0.6 percent, and the minimum amount of evening primrose oil from .0.1 to .0.4 percent. Amount will vary depending on the dietary fat content to maintain levels and ratios of fatty acid groups.

Also, the subject formulations comprise a blend of inorganic minerals and mineral proteinates. The latter form may improve vitamin (and therefore antioxidant) stability since some minerals, such as copper and iron, are pro-oxidants. Because they are metabolized differently than inorganic minerals their availability is also generally higher so the body rather than the stool benefits from the minerals in the diet. Examples thereof include zinc oxide, zinc proteinate, ferrous sulfate, iron proteinate, manganous oxide, copper sulfate, copper proteinate, calcium iodate, sodium selenite, and potassium citrate.

Also, in Nature's Recipe products, fruit and/or vegetable fiber (e.g., tomato pomace) is incorporated as a primary fiber source to maintain normal gastrointestinal function. This is a high quality, moderately fermented fiber in contrast to grain fibers which are more slowly fermented. Additionally, in the Group Specific Formulas, chicory root extract is added which serves as a source of soluble fiber. This material, a source of inulin, has been reported in both humans and pets to promote the growth of beneficial bacteria. Generally, the amounts thereof are sufficient to provide a crude fiber content ranging from 0.5 to 10%, more preferably about 2 to 4% maximum.

Several herbs and spices which are widely used as supplements for humans and pets are added to the subject pet food formulations, including spearmint, ginger, ginseng, ginkgo, parsley, and *Yucca schidigera* extract. These ingredients contribute to the unique aroma and taste of the pet food formulations of the invention. Other herbs and plant materials that may be included comprise milk thistle powder, marigold extract, rosemary, chicory, and cranberry juice extract.

Also, the subject pet food formulations are naturally preserved and are free of added artificial colors and flavors. Chicken is the only meat protein source used and no corn is used in these formulations.

The subject pet food formulations are also designed taking into account feeding studies to ensure nutritional

adequacy. The subject pet food formulations, when properly used, should also satisfy the nutrient ranges established in the AAFCO Nutrient Profiles. This should provide the ultimate assurance of nutritional quality.

Thus, the subject formulations sold under the Nature's Recipe Group Specific Formulas® collectively share some novel ingredients which make them unique relative to competitive products. This includes the use of chicken meat as the primary ingredient, and the only meat-derived material, the use of a more complete antioxidant blend including vitamins, carotenoids, spices and herbs, a unique blend of fats and oils including canola oil, salmon oil and primrose oil, the use of mineral proteinates, a unique fiber blend including tomato pomace and chicory extract, and inclusion of several herbs which are commonly consumed by humans for wellness reasons. The combination of these features provides a formula of both outstanding palatability, digestibility and potential wellness support. The utilization of these diets by the pet exceeds that of any dry diet ever tested at our research facility.

In addition to the common features listed above, the subject breed-specific formulations contain other unique features. These differences include the kibble size and shape, the feeding recommendations, the bag sizes offered (to maintain product freshness), as well as the ingredients and nutrient levels. The variety of shapes and sizes includes a triangle Sporting Diet, square Terrier Diet, rectangular Non-Sporting Diet, almond shaped Hound Diet and round Toy, Working and Herding Diets in variable diameter and thickness appropriate for their sizes.

Specifically, each diet is uniquely different as well with respect to specific nutrient targets (both nutrients listed in the standard AAFCO Nutrient Profile and some potential conditionally essential nutrients which may be of benefit in specific animals), ingredients excluded to prevent intolerances (protein sources and food additives) and functional ingredients. These adjustments have been made because the size and shape outside and metabolism inside differs among breed groups or at least among some lines within these groups. The size and shape of the kibbles were selected based on the range of sizes and mouth configurations within a group in an attempt to encourage consumption. This may help to explain why the diets appear to perform even better in homes where the animal-diet relationship is in harmony compared to a research palatability panel with animals of multiple groups. Feeding directions are also modified both with respect to puppy feeding levels which are restricted in breed groups susceptible to bone and joint abnormalities and for adults in breeds predisposed to obesity. Also, additional choline is added which may reduce carnitine excretion.

Each of the diets incorporates the latest in nutrition and wellness technology. Because these diets are preventative rather than therapeutic in nature and complete with respect to nutrition, there is little harm if animals are not fed the recommended diet. With the common benefits present in these diets, each of these diets provide unsurpassed nutritional delivery compared to competitive products even if the added benefit of breed adjustment is ignored. With this general understanding of the invention, more specific embodiments are described below.

Unique Features of the Sporting Diet Formulation

The Sporting formula contains the highest energy level per cup and the highest percentage of fat calories among the subject breed-specific formulations because of the calorie demands of exercise. Vitamin and mineral fortification as well as choline is also higher in this diet to aid in the processing of nutrients into energy as well as to provide electrolytes. The level of rice in this formula is also higher to support superior digestibility. Exercise increases the generation of free radicals which may be detrimental to the body, so this diet is well fortified with a wide array of antioxidants. The diet is supplemented with sodium bicarbonate (baking soda) to adjust the mineral balance of the diet. This Supplement is widely used in racing horse diets.

Some Irish Setters have been recognized to exhibit an intolerance to gluten which resembles celiac disease in humans. This problem is still not completely understood but thought to result from an intestinal permeability defect in these animals. It is generally handled by avoidance of gluten-containing grains, especially wheat. Barley, rye and oats contain lower levels of gluten and are tolerated by some humans having this condition. However, in the interest of caution, rice is the only grain included in the sporting dog formulation.

Another interesting condition occurs in Cocker Spaniels and likely some Golden Retrievers which have low blood taurine levels that may respond to dietary supplementation. This nutrient is typically added to cat diets but responses in dogs have been unrecognized until recently. It is possible that this nutrient may be of benefit in other breeds as well but research has been limited to this point. The reason for the difference in normal blood levels among breeds is not well known. Several other nutrients/ingredients which act as antioxidant sources are also added including vitamins (such as vitamins E and C), carotenoids, minerals (such as selenium), and herbs (such as rosemary).

Garlic, hawthorn berry powder (for antioxidant activity and cardiac tonicity) and glutamine are also added to this product.

Finally, bone and joint problems have been identified in some of the Sporting breeds, including the Brittany, Irish Setter, Cocker Spaniel and Labrador Retriever. Since the latter two breeds can suffer from obesity as well as adults, feeding reductions of 15% (relative to other breeds) have been recommended for these two breeds. This may have added benefit for bone and joint problems. Additionally, puppy feeding directions are reduced by a similar amount relative to standard for all breeds within this group for similar reasons. Nutrients necessary for bone and joint health including zinc, copper, and vitamin C are added in appropriate amounts and our unique blend of fats and *Yucca schidigera* extract to help manage joint inflammation. Mineral balance is also carefully controlled and glucosamine added.

Based on the foregoing, this formulation represents the most tailored Group-Specific Formula with respect to nutrient diversity, digestibility and incorporation of unique ingredients.

Unique Features of the Working Diet

The Working formulation of the invention contains a high percentage of fat calories to support the calorie demands of exercise. Vitamin and mineral fortification is also higher in this diet to aid in the processing of nutrients into energy as well as to provide electrolytes. The formula comprises a high level of rice to support the high digestibility of this diet. Exercise increases the generation of free radicals which may be detrimental to the body, so this diet is well fortified with a wide array of antioxidants. Choline is also supplemented to aid in fat transport and metabolism. Garlic, hawthorn berry powder and taurine are also added to this product.

An added consideration in the Working diet is maintenance of proper body condition to help manage the additional stress on the skeletal structure. Puppy feeding directions for the entire group have been reduced by 15%

(compared to previous Nature's Recipe feeding directions) to reduce weight gain during growth. Feeding management should also be monitored during the adult period as this is a high energy diet. This also reduces the daily delivery of calcium by a similar extent which may be beneficial for some large breed dogs such as Great Danes which are unable to manage excesses of dietary calcium. No salt is added to manage dietary sodium to the extent possible and dietary acid-base balance is managed with potassium citrate to manage calcium mobilization from bone since meat-based diets and growth itself provide an acid load to the body. Glucosamine is also added for the same reasons as the Herding formulation.

The Working formula represents an excellent choice for Working dogs as well as any breed exposed to moderate stress or exercise level. The diet contains additional beta glucoma fiber from oats and barley. A lot of time and attention went into the development of this diet since the inventors have extensive personal experience in raising and showing breeds in this group.

The Working formula has a generous energy allowance and high digestibility to accommodate the energy needs of this group. As with all Group Specific Formulas, digestibility is quite high. Vitamin and mineral supplementation is also higher in this group to allow it to use this energy.

The Working diet is also managed to the extent possible through dietary means to promote proper cardiovascular function. Dilated cardiomyopathy has been noted in Dobermans, Great Danes and Boxers as well as many other species. In this condition the left ventricle is extremely thin and a heart murmur is identified. The form present in Boxers is different from that in the other breeds listed above. In some animals of this breed, carnitine, a natural compound of the body which promotes fat transfer in the body, has been found to be of benefit in delaying mortality from this condition. Therefore, choline is supplemented to all the Group Specific Formulas to help reduce carnitine excretion from the body. The building blocks of this compound are also supplemented. The managed sodium level present for promotion of bone and joint function may also be of benefit in heart health, and with the level of sodium in meat products, salt addition should be unnecessary. Hawthorn berry powder is also added to this diet. They contain a mixture of bioflavonoids which have antioxidant activity and may reduce blood pressure, Potassium supplemental as is done in this formula may also have modest benefits in this regard.

The antioxidants present in all of the diets including vitamins, minerals, carotenoids, spices and herbs as well as the omega 3 and GLA oils added to these diets for inflammation management should also assist in prevention of problems.

Many antioxidant nutrients have been found to be of some benefits in cardiomyopathies of different species, including selenium, a mineral, in livestock and taurine, an amino acid-like compound in cats and some dog breeds such as Cocker spaniels and Golden Retrievers. As a precaution, the Working diet is supplemented with both selenium and taurine as extensive research has not been conducted in all breed groups and the nutrients will do no harm at the level of supplementation utilized.

Unique Features of the Non-sporting Diet

The Non-Sporting formula is highly digestible but contains a managed level of protein and fat calories. These characteristics make this diet an excellent choice for mixed breed dogs, for spayed and neutered pets or for pet owners desiring to control protein and fat calories. The mineral balance is adjusted by the use of sodium hexametaphosphate. In fact, the Non-Sporting formula, because of its high energy level, without excessive use of protein or fat, serves as an excellent all-purpose diet both for Non-Sporting and mixed breed dogs.

The Non-Sporting group probably has as wide a diversity of pet types as any group. The diet designed for them is a moderate protein, moderate calorie diet which serves as our recommendation for mixed breed dogs of normal activity. For those engaged in extreme activity, the Sporting formula would then be recommended.

The most well-recognized problem of this group which is diet responsive is urate crystals in Dalmatians. Because of the extreme dietary adjustments required for this breed, we generally consider this condition to be a candidate for a veterinary medical diet. In fact, many pets of this breed have been fed Nature's Recipe Vegetarian Canine Formula as it has several attributes thought to be beneficial for prevention of this condition. This breed was not ignored in the dietary formulation of the Non-Sporting formula, however. The total protein level in this formula is managed because it is this nutrient group which is ultimately the source of uric acid. Additionally, the meat protein source is meat muscle tissue rather than meat by-products since the latter is higher in nucleic acids.

As stated previously, the Vegetarian Formula would be better still in this respect. Additionally, this condition is best addressed in a more alkaline urine and the Non-Sporting formula, because of the specific mineral balance associated with ingredients such as sodium hexametaphosphate, should promote a more alkaline diet than any of the other Group Specific Formulas. Although certainly not proven scientifically, chicory root has been used in humans for gout to increase uric acid excretion, likely resulting on its effects on the liver or as a diuretic in the kidney.

Skin and hair coat problems have been noted in several breeds including the Chinese Shar Pei, the Chow Chow and the Miniature Poodle. This problem is also accounted for in the subject breed-specific formulations. In addition to a generous supply of vitamins (B-vitamins, vitamin A) and minerals (zinc and copper in proteinate form which is more available for deposition in hair), the Group Specific Formulas incorporate the latest in fatty acid supplementation technology available today. This involves a careful balance of total omega-6 and omega-3 fatty acids (ratio 4-11) as well as supplementation of a balance of short and long chain compounds in these major classifications to facilitate inflammation management. This is the reason for the supplementation of evening primrose oil and salmon oil in addition to canola oil in the subject formulations. This blend provides insurance for pets which may have low enzyme activities.

Since most true food allergens are proteins, management of dietary protein is important. This is why the total protein level is managed and a single source (chicken) is used in our formulations. Additionally, as with all Nature's Recipe products, natural preservatives and flavors are used and no colorants are added to the subject formulations. Since proteins can sometimes be found in unrefined fats and oils typically used in the pet food industry, we use highly refined human grade oils that are shipped under a nitrogen blanket to ensure freshness and retard oxidation.

A final dietary adjustment made only in the Non-Sporting formula is the use of agents to help control tartar accumulation. Some breeds including Poodles and Bichon Frise are thought to accumulate tartar at greater rates than others and/or suffer from a higher rate of periodontal disease. The present formulations comprise a patented tartar control agent

called sodium hexametaphosphate which has been licensed exclusively to Heinz. It has been reported in peer-reviewed journals that this agent reduces the rate of tartar accumulation 80% when added in a complete meal. Until now this ingredient has only been added to pet treats. However, with dentists telling us to brush after every meal it was decided that it would be beneficial to incorporate such ingredient in the subject formulations so it would be consumed more frequently. Other ingredients such as bromelain and cranberry extract have also been reported to improve oral health and promote healing and are additionally included in this formulation.

Unique Features of The Herding Diet

The Herding formula is an excellent diet which provides a high energy level without excessive use of fat. It has been tested with police dogs in training, so it should serve the needs of pets in less stressful environments.

More specifically, some breeds in the herding group such as German Shepherds suffer from a gastrointestinal immune deficiency which manifests itself through chronic, intermittent diarrhea. Additionally, many of the breeds in this group as well as Working and Sporting group suffer from numerous bone and joint abnormalities, including hip and elbow dysplasia, panosteitis, and degenerative joint disease.

Since most breeds can experience diarrhea on occasion, particularly in response to stress, all Group Specific Formulas share a combination of ingredients to help maintain gastrointestinal function. One of these is the inclusion of high levels of rice which, in addition to being highly digestible, contain compounds which actually inhibit intestinal secretions. The diets also contain spearmint and ginger, thought to inhibit nausea which may lead to gastrointestinal disturbances. They also contain a blend of fruit and/or vegetable fibers (e.g., tomato pomace), chicory extract and *Yucca schidigera* extract which provide unique benefits relative to gastrointestinal health. The specific fibers used are moderately fermented similar to beet pulp but of more health origin (additionally provide antioxidants). While fiber used to be thought of as a filler, it is now recognized that fiber is important for intestinal health. Chicory extract contains inulin, the parent compound of fructooligosaccharides (FOS) found in IVD Select Care formulas and more recently in EukanuBA diets. This compound is termed a "prebiotic" in that it can be utilized by beneficial microorganisms like bifidobacteria and lactobacillus but not be harmful ones including Salmonella, Clostridia and *E. coli*. Simply put, it is food for good bugs. The chicory extract is a natural extract of the chicory root while the FOS used by EukanuBA is a fermentation product of sugar. Both chicory extract and *Yucca schidigera* extract act to bind bacterial toxins and ammonia and help to reduce stool odor. This effect has been noted both in humans and pets.

The unique fats and oils used in this product line also help to control inflammation both in gastrointestinal disturbances and in joint inflammation. Zinc in both inorganic and proteinate form is provided to assist in repair of intestinal cell damage. High levels of zinc have been used in many species with intestinal disorders with excellent results.

While these ingredient adjustments provide excellent protection of gastrointestinal function, the Herding diet adds other unique ingredients to protect this particular organ. One way is through the use of microbial cultures (probiotics or DFM) which provide beneficial organisms to the pet. This concept is similar to yogurt. They are added after the extrusion process to protect them from heat damage. In this particular formula a combination of *Lactobacillus acidophilus, Bacillus subtilis* and *Entercococcus faecium* is used because of their complementary action. Microbial cultures serve as a source of enzymes to help digest food, competitively exclude harmful bacteria, and synthesize various B vitamins and antimicrobial compounds. The combination of prebiotics and probiotics in the same product as a 1–2 punch has been termed "synbiotics" and is the most current trend in progressive yogurt cultures. In addition to the enzymes in these microbial cultures, the Herding diet contains bromelain, an extract of pineapple which contains a complex of several protein-digesting enzymes to complement the pet's natural digestive capabilities. A final ingredient which is supplemented is glutamine. This compound is a natural component of the body which is the primary fuel source for the intestinal cells and in particular immune cells of the gastrointestinal tract. It plays a similar role to butyric acid, provided by fiber fermentation, for health of cells of the colon. Although it may not help healthy animals at rest, it may be of benefit in stress conditions including weaning to prevent muscle breakdown as a source of this amino acid. In addition to the addition of these many functional ingredients, the calories from fat are reduced in the Hound diet.

The total calories are similar to many premium diets because of the high digestibility of this diet. The reason for the reduced fat is that, in the presence of gastrointestinal disturbances, fat digestion is most compromised and results in stool malodor from bacterial fermentation of fat. The fat management helps reduce the harm caused by intestinal dysfunction. The level of vitamin and trace mineral supplementation is higher in this diet than some of other Group Specific Formulas to help utilization of energy, particularly in pets under stress.

As indicated previously, dietary adjustments are also made to ensure proper bone and joint function. Feeding instructions have been altered to a 15% reduction relative to groups with minimal bone and joint problems during the puppy phase and breeds with a propensity for obesity such as Collies and Shetland Sheepdogs reduced by a similar amount during the adult phase to minimize weight burden on the joints. Daily calcium consumption is also managed by this dietary restriction as it appears that the puppy of at least some large breeds are unable to regulate its calcium absorption. Sodium is also restricted to help minimize calcium mobilization from the bone as urinary sodium and calcium excretion run parallel to one another. Functional ingredients such as glucosamine are added to enhance proteoglycan synthesis and prevent its destruction. Aspirin and ibuprofen suppress pain but in fact suppress proteoglycan synthesis, preventing the body to self repair damage. Potassium citrate is used to adjust the mineral (acid-base) balance of the body and hopefully enhance bone mineral deposition. Research with several species including cats has indicated that diets with a low mineral balance (more acid in nature) cause bone demineralization. This would be particularly harmful in growing animals since bone formation itself generates acid. Bromelain has also been found to be of some benefit in joint disorders, perhaps through some yet unknown indirect anti-inflammatory mechanism.

As with intestinal disorders, all diets contain some dietary components to promote strong bones and joint function including the fatty acids listed above as well as potentially the yucca extract to control joint inflammation, manganese supplementation (cofactor in enzymes in chondroitin synthesis), zinc supplementation (protein and DNA synthesis), iron and vitamin C (for the hydroxylation of proline during collagen formation) and copper (for cross-linking of collagen molecules to provide cartilage strength)

as well as biotin and choline (for proteoglycan formation and aggregation). The ingredients listed above are added in the diets specifically designed for breed groups with a high propensity of bone and joint problems, including Herding dogs.

Unique Features of the Terrier Diet

The diet for this group has a fairly high protein level and high fat level relative to its total calories to meet the needs of the active terrier group. For breeds such as Cairn and Scottish Terriers, which may be "easy keepers" adult feeding directions recommend a 15% reduction relative to other breeds within this group.

A well-recognized problem within this group is a liver copper storage disease present in Bedlington Terriers and West Highland Terriers. It is estimated that in the former breed both England and the United States that approximately 25% of the animals are affected and an additional 50% are carriers as it is transmitted as an autosomal recessive gene. This condition bears some resemblance to Wilson's disease in humans which has an estimated worldwide incidence of approximately 1 in 30,000. This condition is characterized by a toxic accumulation of copper in the liver and brain, resulting in tremors, psychiatric disturbances, and liver degeneration in humans. This condition is typically treated by a combination of dietary copper restriction and additional zinc supplementation.

In humans, liver consumption is not recommended although recent studies have suggested that while copper in beef liver is highly available, that in pork is not. Zinc supplementation reduces the retention of copper in the body and thus has an indirect beneficial effect. Level of copper supplementation is lower and zinc higher in the Terrier formula than in any of the other Group Specific Formulas. Milk thistle has been added as a complementary ingredient to help maintain liver health. It contains a group of compounds called the silymarin complex of bioflavonoids. It both helps bind toxins to complement healthy liver function stimulates the production of several potent antioxidants (glutathione and superoxide dismutase) to protect the liver cells from oxidation and finally is thought to stimulate protein synthesis in the liver to enhance repair. This herb has been the subject of hundreds of clinical studies and has been supplemented in Europe for humans with alcoholism, chronic hepatitis and liver cirrhosis.

Unique Features of the Toy Diet

The Terrier formula is highly digestible but contains a managed level of protein and intermediate fat calories, ideal for the needs of active Toy breeds.

More specifically, the Toy formula is managed in protein and contains a high proportion of fat calories relative to several Group Specific Formulas. The rationale behind this is that some of the dogs in this group such as Pugs and Pekinese are brachycephalic and are somewhat intolerant of heat stress. Digestion and metabolism of protein generates more heat. Also, digestion and metabolism of fat generates less heat than carbohydrates. Consequently, these adjustments of protein and fat may be beneficial, particularly when pets are in warm environments or exercising outdoors in the summer. Additionally, sodium bicarbonate and potassium chloride are included to help encourage water consumption and to provide electrolytes. Chromium (from yeast) is incorporated since it appears in some species to reduce cortisol levels present when animals are exposed to stressors.

Additionally, it has been noted that some breeds such as Silky Terriers and Yorkshire Terriers are susceptible to diabetes. Certainly energy management to maintain optimum body condition is useful. As noted, chromium (from yeast) is supplemented, as well as niacin in this diet. These nutrients are thought to co-participate in a complex known as glucose tolerance factor which is thought to act to improve insulin sensitivity in the body. Additionally, results of recent research suggest that barley has a lower glycemic index in pet than other common grains such as corn or wheat or especially rice. This diet has the highest amount of barley among this line of products. It should be noted that such supplementation acts as a complement, not replacement to insulin administration (frequently required in canine diabetes).

Several breeds including the Yorkshire Terrier, Toy Poodle, Pomeranian, Shih Tzu, Affenpinscher and Pekinese can suffer from oxalate urinary calculi which are responsive to diet mineral adjustment away using alkalizing minerals such as sodium bicarbonates. Additionally, unlike cats, many of the stones in dogs are complicated by urinary tract infections. Cranberry extract is added to this formulation. This latter ingredient is widely utilized in humans for urinary tract infections and is thought to prevent bacterial adhesion to the urinary tract.

The small size of the Toy formula is also designed based on the relatively small mouth size of Toy breeds.

Unique Features of the Hound Diet

The Hound formula is an excellent diet which provides a high energy level without excessive use of fat. It has a very unique shape relative to any commercial diet. Specifically, the Hound formulation contains a high protein level but managed fat level. Many of the hound breeds are extremely lean and therefore would have a higher protein requirement. Some, however, such as Basset Hounds, Dachshunds and Beagles can become overweight with age and so a caloric reduction of 15% is similarly recommended for these breeds. Additionally, because of their relative body dimensions (short and long), disc disease can occur in Dachshunds and maintaining a lean body is important to reduce the stress on the vertebrae.

Similar to some of the Herding dogs, several animals in the Hound group including the Basenji, Beagle and Whippet can experience chronic, intermittent diarrhea. In the case of the Beagle it may result from a deficiency of intestinal immunity which resembles that in German Shepherds. To attempt to counteract this condition, all of the various ingredients mentioned previously for the Herding diet are added to the Herding formulation. This includes the addition of a high level of rice, a combination of probiotics (microbial cultures) and prebiotics (chicory root extract-inulin) which is termed synbiotics, inclusion of bromclain to assist in digestion, spearmint and ginger to soothe the stomach, glutamine and zinc to promote intestinal cell repair, and a blend of soluble and insoluble fibers to optimize intestinal environment. Additionally, garlic is thought to have some natural ability to inhibit growth of pathogenic organisms. The unique fat blend should help moderate intestinal inflammation.

In contrast to the Toy formula discussed earlier, the Hound diet is formulated with a more acid mineral balance as some breeds in this group such as Basset Hounds are more predisposed to stravite rather than oxalate urinary calculi. Cranberry extract is again added as a safeguard since bacterial infections can complicate this condition. Potassium citrate is still added to help complex calcium and reduce risked calcium oxalate stones as well. It is our belief that a moderate diet containing a mineral balance promoting a moderately acid urine combined with magnesium and citrate is a more effective approach to urinary tract health than an extreme diet which helps with one type of stone such as

stravite but increases susceptibility to the other (such as calcium oxalate). From the standpoint of dogs susceptible to stravite stones, this diet should provide the most acidic urine among this diet line and is therefore likely to be most effective.

In order to further facilitate an understanding of the invention, the following examples are provided.

EXAMPLE 1

Non-Sporting Dog Formulation

A non-sporting dog formulation was formulated to provide for managed protein, moderate energy, highly digestible diet using wholesome grains like ground rice, rolled oats and cracked pearled barley. This product is formulated to maximize the benefits of good nutrition to all parts of the body, including the gastrointestinal tract. The formula includes Spearmint as well as Bromelain and is formulated to help maintain oral health. Herbs such as Ginkgo, Ginseng and Parsley assist in overall good pet health. This unique combination of ingredients provides excellent taste and 100% complete and balanced nutrition for all life stages.

This unique formulation for the NON-SPORTING GROUP breeds also contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

The specific ingredients in this formulation are as follows: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Bone Phosphate, Tomato Pomace, Brewers Dried Yeast, Sodium Hexametaphosphate, Chicory Root Extract, Potassium Chloride, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Sodium Chloride, Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Potassium Citrate, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, Bromelain, and Marigold Extract.

Animal feed testing according to the procedures of the Association of American Feed Control Officials have shown that this formulation has a complete and balanced diet for all stages of life. The analysis of this formulation is provided below.

ANALYSIS	
CRUDE PROTEIN	22.0% MINIMUM
CRUDE FAT	12.0% MINIMUM
CRUDE FIBER	4.0% MAXIMUM
MOISTURE/	10.0% MAXIMUM
CALCIUM	1.1% MINIMUM
PHOSPHORUS	0.85% MINIMUM
OMEGA-6 FATTY ACIDS	2.75%* MINIMUM
OMEGA-3 FATTY ACIDS	0.3%* MINIMUM
GAMMA LINOLENIC ACID	0.02%* MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

The kibble shape of this formulation was specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Non-Sporting Diet is shaped as a rectangle with length nearly 3 times the width. It is an extremely narrow kibble approximately 7 mm wide. This unique shape encourages chewing which may participate in its tartar control effects.

American Eskimo Dog	Dalmatian	Poodle
Bichon Frise	Finnish Spitz	Schipperke
Boston Terrier	French Bulldog	Shiba Inu
Bulldog	Keeshond	Tibetan Spaniel
Chinese Shar Pei	Lhasa Apso	Tibetan Terrier
Chow Chow		

As discussed, the nutritional requirements of dogs vary according to breed, age, size, activity and environment. From weaning to six months of age, feed the recommended amount in 3 feedings per day. Over six months of age, feed the recommended amount in 2 feedings per day. It is recommended that the subject formulation be consumed based on the following recommended guidelines.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6–12 Months	Greater Than 12 Months †
3–10	1¼–2¾ Cups	⅞–2 Cups	⅝–1¼ Cups
10–15	2½–3½ Cups	2–2⅝ Cups	1⅜–1¾ Cups
15–25	3½–5 Cups	2⅝–3¼ Cups	1¾–2½ Cups
25–50	5–8 Cups	3¼–6 Cups	2½–4 Cups
50–75	8–10⅝ Cups	6–7⅞ Cups	4–5¼ Cups

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
† For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.

EXAMPLE 2

Toy Dog Formulation

As discussed above, the formulation for the TOY GROUP breeds contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including

Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

A Toy dog formulation was produced comprising the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Bone Phosphate, Chicory Root Extract, Potassium Chloride, Sodium Bicarbonate, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Cranberry Juice Concentrate, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract. The analysis of this formulation is provided below.

ANALYSIS		
CRUDE PROTEIN	22.0%	MINIMUM
CRUDE FAT	12.0%	MINIMUM
CRUDE FIBER	4.0%	MAXIMUM
MOISTURE	10.0%	MAXIMUM
CALCIUM	1.0%	MINIMUM
PHOSPHORUS	0.80%	MINIMUM
NIACIN	100 mg/kg	MINIMUM
CHROMIUM	2.0 mg/kg*	MINIMUM
OMEGA–6 FATTY ACIDS	3.0%*	MINIMUM
OMEGA–3 FATTY ACIDS	0.3%*	MINIMUM
GAMMA LINOLENIC ACID	0.03%*	MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

As with the previous formulation, animal feeding tests using the procedures of the Association of American Feed Control Officials indicate that this formulation provides complete and balanced nutrition for all stages of life.

Again, for this formulation, the kibble shape was specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Toy diet is a small cylindrical kibble of diameter 8.5 mm, the smallest of the round kibble in this line. This smaller round kibble fits nicely in the mouth of many Toy breeds with small square mouths.

Affenpinscher	Japanese Chin	Pomeranian
Brussels Griffon	Maltese	Toy Poodle
Cavalier King Charles Spaniel	Toy Manchester Terrier	Pug
Chihuahua	Miniature Pinscher	Shih Tzu
Chinese Crested	Papillon	Silky Terrier
English Toy Spaniel	Pekingese	Yorkshire Terrier
Italian Greyhound		

Feeding Guidelines

It is recommended that from weaning to six months of age, the recommended amount be fed three times per day. Over six months of age, it is recommended that this be reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6–12 Months	Greater Than 12 Months †‡
2–5	⅛–1½ Cups	⅛–1⅛ Cups	⅛–¾ Cups
5–10	1½–2½Cups	1⅛–1⅞ Cups	¾–1¼ cups
10–15	2½–3¼ Cups	1⅞–2⅜ Cups	1¼–1⅝ Cups
15–25	3¼–4 ½ Cups	2⅜–3⅜ Cups	1⅝–2¼ Cups

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.

†For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.

EXAMPLE 3

Terrier Dog Formulation

As discussed supra, the TERRIER GROUP breed formulation contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

Based on the foregoing, a Terrier dog formulation comprising the following ingredients and analysis was produced: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Potassium Chloride, Bone Phosphate, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Sodium Chloride, Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Milk Thistle Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract. The analysis of this formulation is provided below.

ANALYSIS	
CRUDE PROTEIN	25.0% MINIMUM
CRUDE FAT	12.0% MINIMUM
CRUDE FIBER	4.0% MAXIMUM
MOISTURE	10.0% MAXIMUM

-continued

ANALYSIS	
CALCIUM	1.0% MINIMUM
PHOSPHORUS	0.85% MINIMUM
COPPER	7.5 mg/kg MINIMUM
ZINC	300 mg/kg MINIMUM
OMEGA-6 FATTY ACIDS	3.0%* MINIMUM
OMEGA-3 FATTY ACIDS	0.3%* MINIMUM
GAMMA LINOLENIC ACID	0.03%* MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

Similarly, animal feeding tests using the procedures of the Association of American Feed Control Officials have shown that NATURE'S RECIPE TERRIER DOGS GROUP SPECIFIC FORMULA provides a complete and balanced nutrition for all stages of life.

The kibble shape of this formulation was again specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Terrier diet has a square shape of intermediate size 11.5 mm per side. This shape resembles the square shape of the skull of the Airedale Terrier, Irish Terrier, and Welsh Terrier.

Airedale Terrier	Wire Fox Terrier	Norwich Terrier
American Staffordshire Terrier	Irish Terrier	Scottish Terrier
Australian Terrier	Kerry Blue Terrier	Sealyham Terrier
Bedlington Terrier	Lakeland Terrier	Skye Terrier
Border Terrier	Standard Manchester Terrier	Soft Coated Wheaten Terrier
Bull Terrier	Miniature Bull Terrier	Staffordshire Bull Terrier
Cairn Terrier	Miniature Schnauzer	Welsh Terrier
Dandie Dinmont Terrier	Norfolk Terrier	West Highland White Terrier
Smooth Fox Terrier		

Recommended Feeding Instructions

It is recommended that from weaning to six months of age, the recommended amount be given in three feedings per day. Over six months of age, it is recommended that the amount be reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6 –12 Months	Greater Than 12 Months †‡
3–10	1½–2½ Cups	⅞–1⅞ Cups	½–1¼ Cups
10–15	2½–4Cups	1⅞–2⅞ Cups	1¼–1¾ Cups
15–25	4–4⅞ Cups	2½–3½ Cups	1¾–2¼ cups
25–50	4⅞–7¼ Cups	3½–5½ Cups	2¼–3¾ Cups
50–75	7⅛–9¾ Cups	5½–7¼ Cups	3¾–4¾ Cups

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
†For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.
‡For Cairn and Scottish Terriers feed 15% less than the amount indicated.

EXAMPLE 4

Working Dog Formulation

As discussed supra, the WORKING GROUP breed formulation contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

Based on the foregoing, a Working Group Formulation was designed comprising the following ingredients and analysis: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Potassium Citrate, Glucosamine Hydrochloride, Hawthorn Berry Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Taurine, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Garlic Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract. The analysis of this formulation is provided below.

ANALYSIS	
CRUDE PROTEIN	24.0% MINIMUM
CRUDE FAT	13.0% MINIMUM
CRUDE FIBER	4.0% MAXIMUM
MOISTURE	10.0% MAXIMUM
CALCIUM	1.1% MINIMUM
PHOSPHORUS	0.85% MINIMUM
SELENIUM	0.4 mg/kg MINIMUM
TAURINE	0.05%* MINIMUM
OMEGA-6 FATTY ACIDS	3.0%* MINIMUM
OMEGA-3 FATTY ACIDS	0.4%* MINIMUM
GAMMA LINOLENIC ACID	0.05%* MINIMUM

*Not recogtiized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

Again, animal feeding tests using the procedures of the Association of American Feed Control Officials substantiate that the above NATURE'S RECIPE WORKING DOGS GROUP SPECIFIC FORMULA provides complete and balanced nutrition for all stages of life.

Again, the kibble shape of this formulation was specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Working diet is a flattened disc kibble 12 mm in diameter. Being intermediate in size and thickness, it suits the diversity of mouth configurations of this group.

Akita	Great Dane	Portuguese Water Dog
Alaskan Malamute	Great Pyrenees	Rottweiler
Bernese Mountain Dog	Great Swiss Mountain Dog	Saint Bernard

-continued

Boxer	Komondor	Samoyed
Bullmastiff	Kuvasz	Siberian Husky
Doberman Pinscher	Mastiff	Standard Schnauzer
Giant Schnauzer	Newfoundland	

Recommended Feeding Instructions

From weaning to six months of age, feed the recommended amount in three feedings per day. Over six months of age, the recommended amount is reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6–12 Months	Greater Than 12 Months †
3–10	1–2⅛ Cups	⅛–1⅜ Cups	⅛–1⅛ Cups
10–20	2⅛–3½ Cups	1⅜–2⅝ Cups	1⅛–2 Cups
20–50	3½–6⅜ Cups	2⅝–4⅜ Cups	2–3¾ Cups
50–100	6⅜–10⅛Cups	4⅜–7⅛ Cups	3¾ Cups–6 Cups
Over 100			Add ⅜ cup for each 10 lbs.

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
†For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.

EXAMPLE 5

Hound Dog Formulation

As discussed supra, the HOUND GROUP breed formulation also contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

Based on the foregoing, a formulation designed for Hound Dogs comprising the following ingredients and analysis was made: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Potassium Citrate, *Lactobacillus acidophilus, Enterococcus faecium, Bacillus subtilis* Fermentation Extract, *Aspergillus oryzae* Fermentation Extract, *Aspergillus niger* Fermentation Extract, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Cranberry Juice Concentrate, Siberian Ginseng Extract, Parsley Seed Oil Powder, Glutamine, Ginger Extract, Bromelain, and Marigold Extract. The analysis of this formulation is provided below.

ANALYSIS

CRUDE PROTEIN	26.0% MINIMUM
CRUDE FAT	10.0% MINIMUM
CRUDE FIBER	4.0% MAXIMUM
MOISTURE	10.0% MAXIMUM
CALCIUM	1.0% MINIMUM
PHOSPHORUS	0.8% MINIMUM
OMEGA-6 FATTY ACIDS	2.75%* MINIMUM
OMEGA-3 FATTY ACIDS	0.2%* MINIMUM
GAMMA LINOLENIC ACID	0.02%* MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

Animal feeding tests using the procedures of the Association of American Feed Control Officials substantiate that NATURE'S RECIPE HOUND DOGS GROUP SPECIFIC FORMULA provides a complete and balanced nutrition for all stages of life.

Again, the kibble shape of this formulation was specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Hound diet can best be described as an almond or tear drop shape. It has a length at longest point slightly shorter than the Non-Sporting diet. This shape perfectly conforms to breeds of this group such as the Afghan Hound.

Afghan Hound	American Foxhound	Otterhound
Basenji	English Foxhound	Petit Basset Griffon Vendeen
Basset Hound	Greyhound	Pharaoh Hound
Beagle	Harrier	Rhodesian Ridgeback
Black & Tan Coonhound	Ibizan Hound	Saluki
Bloodhound	Irish Wolfhound	Scottish Deerhound
Borzoi	Norwegian Elkhound	Whippet
Dachshund		

Recommended Feeding Instructions

From weaning to six months of age, feed the recommended amount in three feedings per day. Over six months of age, feed the recommended amount is reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6 –12 Months	Greater Than 12 Months †‡
3 –10	1⅛–2⅝Cups	⅛–2 Cups	⅜–1¾ Cups
10–20	2⅝–4½ Cups	2–3¼ Cups	1¾–2⅛ Cups
20–50	4½–7⅝ Cups	3⅛–5⅞ Cups	2⅛–3⅜ Cups
50–100	7⅝–12⅜ Cups	5⅞–9⅜ Cups	3⅜–6¼ Cups
Over 100			Add 3/8 cup for each 10 lbs.

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
†For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.
‡For Basset Hounds, Dachshunds and Beagles feed 15% less than the amount indicated.

EXAMPLE 6

Herding Dog Formulation

As discussed supra, the HERDING GROUP breed formulation contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

Based on the foregoing, a formulation adapted for Herding Dogs comprising the following ingredients and analysis was made: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Potassium Citrate, *Lactobacillus acidophilus, Enterococcus faecium, Bacillus subtilis* Fermentation Extract, *Aspergillus oryzae* Fermentation Extract, *Aspergillus niger* Fermentation Extract, Glucosamine Hydrochloride, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Panax Ginseng Root Powder, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Chinese Ginseng Root, Parsley Seed Oil Powder, Ginger Extract, Glutamine, Bromelain, Marigold Extract. The analysis of this specific formulation is provided below.

ANALYSIS	
CRUDE PROTEIN	24.0% MINIMUM
CRUDE FAT	10.0% MINIMUM
CRUDE FIBER	4.0% MAXIMUM
MOISTURE	10.0% MAXIMUM
CALCIUM	1.0% MINIMUM
PHOSPHORUS	0.8% MINIMUM
OMEGA-6 FATTY ACIDS	2.75%* MINIMUM
OMEGA-3 FATTY ACIDS	0.2%* MINIMUM
GAMMA LINOLENIC ACID	0.02%* MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

Animal feeding tests using the procedures of the Association of American Feed Control Officials substantiate that NATURE'S RECIPE HERDING DOGS GROUP SPECIFIC FORMULA provides a complete and balanced nutrition for all stages of life.

The kibble shape of this formulation was again specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Herding diet somewhat resembles the Working diet which relates to the fact that these breeds were at one time in a single group. The Herding diet has a smaller diameter (10.5 mm vs. 12 mm) but is thicker (8 mm vs. 6 mm) than the Working formula.

Australian Cattle Dog	Border Collie	Old English Sheepdog
Australian Shepherd	Bouvier Des Flandres	Puli
Bearded Collie	Briard	Shetland Sheepdog
Belgian Malinois	Canaan	Cardigan Welsh Corgi
Belgian Sheepdog	Collie	Pembroke Welsh Corgi
Belgian Tervuren	German Shepherd Dog	

Recommended Feeding Guidelines

From weaning to six months of age, feed the recommended amount in three feedings per day. Over six months of age, the recommended amount is reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6–12 Months	Greater than 12 Months †‡
3–10	1–2¼ Cups	¼–1⅝ Cups	⅝–1¼ Cups
10–20	2⅛–3½ Cups	1⅝–2⅝ Cups	1¼–2 Cups
20–50	3–6⅛ Cups	2⅛–4¾ Cups	2–3¾ Cups
50–100	6⅛–10¼ Cups	4¾–7⅝ Cups	3¾ Cups–6 Cups
Over 100			Add ¾ cup for each 10 lbs.

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
† For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.
‡ For Collies and Shetland Sheepdogs feed 15% less than the amount indicated.

EXAMPLE 7

Sporting Dog Formulation

As discussed supra, the SPORTING GROUP breed formula contains a blend of Canola Oil, Salmon Oil and Evening Primrose Oil, a source of GLA (gamma linolenic acid), naturally preserved with Tocopherols. This, in combination with Chicken, provides an optimum balance from the full spectrum of polyunsaturated fatty acids including Omega 6 and Omega 3 for maintaining healthy skin and hair coat. In addition, this formula contains a special blend of antioxidant vitamins and minerals to prevent or neutralize the damaging effects of free radicals.

Based on the foregoing, a formulation designed for Sport Group Dogs comprising the following ingredients and analysis was produced: Chicken, Ground Rice, Chicken Meal, Natural Flavor, Canola Oil (Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid), Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Potassium Chloride, Sodium Bicarbonate, Vitamins (Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid [Source of Vitamin C], d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride [Vitamin B_6], Folic Acid, Menadione Sodium Bisulfite Complex [Source of Vitamin K activity], Biotin, Vitamin B_{12} Supplement), Salmon Oil, Evening Primrose Oil, Minerals (Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, Sodium Selenite), Glucosamine Hydrochloride, Hawthorn Berry

Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Taurine, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parley Seed Oil Powder, Ginger Extract, Glutamine, and Marigold Extract. The analysis of this formulation is provided below.

ANALYSIS	
CRUDE PROTEIN	25.0% MINIMUM
CRUDE FAT	15.0% MINIMUM
CRUDE FIBER	3.0% MAXIMUM
MOISTURE	10.0% MAXIMUM
CALCIUM	1.0% MAXIMUM
PHOSPHORUS	0.8% MAXIMUM
SODIUM	0.40% MINIMUM
TAURINE	0.05%* MINIMUM
OMEGA-6 FATTY ACIDS	30.%* MINIMUM
OMEGA-3 FATTY ACIDS	0.4%* MINIMUM
GAMMA LINOLENIC ACID	0.025%* MINIMUM

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profile.

Animal feeding tests using the procedures of the Association of American Feed Control Officials also indicate that the above NATURE'S RECIPE SPORTING DOGS GROUP SPECIFIC FORMULA provides a complete and balanced nutrition for all stages of life.

The kibble shape of this formulation was again specifically designed based on size, weight, and breed of dogs belonging to this particular group, as were the designated feeding guidelines below. The Sporting Diet has a unique triangle shape, well suited to the prevalence of pointed jaws of the breeds in this group.

Brittany	Labrador Retriever	English Springer Spaniel
Pointer	English Setter	Field Spaniel
German Shorthaired Pointer	Gordon Setter	Irish Water Spaniel
German Wirehaired Pointer	Irish Setter	Sussex Spaniel
Chesapeake Bay Retriever	American Water Spaniel	Welsh Springer Spaniel
Curly-Coated Retriever	Cocker Spaniel	Vizsla
Flat-Coated Retriever	Cocker Spaniel	Weimaraner
Golden Retriever	English Cocker Spaniel	Wirehaired Pointing Griffon

Recommended Feeding Guidelines

From weaning to six months of age, feed the recommended amount in three feedings per day. Over six months of age, the recommended feeding amount is reduced to two feedings per day.

Daily Feeding Guidelines

Weight of Dog (lbs.)	Weaning to 6 Months	6–12 Months	Greater Than 12 Months †‡
3–10	¼–1⅛ Cups	¼–1⅜ Cups	½–1⅓ Cups
10–20	1⅛–2 Cups	1⅜–2¼ Cups	1⅓–1¾ Cups
20–50	2–5⅝ Cups	2¼–4¼ Cups	1¾–3¼ Cups
50–100	5⅝–9 Cups	4¼–6¼ Cups	3¼–5¼ Cups Add ⅜ cup for each 10 lbs.
Over 100			

Measurements are based on a standard 8 oz. cup which equal approximately 3.5 oz. of dry kibble.
† For gestation and lactation feed as much as 3 times the amount indicated in 3 feedings per day.
‡ For Labrador Retriever and Cocker Spaniels feed 15% less than the amount indicated.

What is claimed is:

1. A pet food formulation which is adapted for non-sporting dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Bone Phosphate, Tomato Pomace, Brewers Dried Yeast, Sodium Hexametaphosphate, Chicory Root Extract, Potassium Chloride, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, Biotin, and Vitamin B_{12} Supplement, Sodium Chloride Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Potassium Citrate, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, Bromelain, and Marigold Extract.

2. A pet food formulation which is adapted for toy dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Bone Phosphate, Chicory Root Extract, Potassium Chloride, Sodium Bicarbonate, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, Biotin, and Vitamin B_{12} Supplement, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Cranberry Juice Concentrate, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract.

3. A pet food formulation which is adapted for terrier dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley,

Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Potassium Chloride, Bone Phosphate, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, and Biotin, and Vitamin B_{12} Supplement, Sodium Chloride, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Milk Thistle Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract.

4. A pet food formulation which is adapted for working dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, and Biotin, Vitamin B_{12} Supplement, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Potassium Citrate, Glucosamine Hydrochloride, Hawthorn Berry Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Taurine, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Garlic Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, and Marigold Extract.

5. A pet food formulation which is adapted for hound dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, and Biotin, Vitamin B_{12} Supplement, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Potassium Citrate, *Lactobacillus acidophilus, Entercococcus faecium, Bacillus subtilis* Fermentation Extract, *Aspergillus oryzae* Fermentation Extract, *Aspergillus niger* Fermentation Extract Ginkgo Biloba Extract, *Yucca schidigera* Extract, Garlic Powder, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Cranberry Juice Concentrate, Siberian Ginseng Extract, Parsley Seed Oil Powder, Glutamine, Ginger Extract, Bromelain, and Marigold Extract.

6. A pet food formulation which is adapted for herding dogs that comprises the following ingredients: Chicken, Ground Rice, Rolled Oats, Chicken Meal, Cracked Pearled Barley, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Bone Phosphate, Potassium Chloride, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, and Biotin, Vitamin B_{12} Supplement, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Potassium Citrate, *Lactobacillus acidophilus, Entercococcus faecium, Bacillus subtilis* Fermentation Extract, *Aspergillus oryzae* Fermentation Extract, *Aspergillus niger* Fermentation Extract, Glucosamine Hydrochloride, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Panax Ginseng Root Powder, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Chinese Ginseng Root, Parsley Seed Oil Powder, Ginger Extract, Glutamine, Bromelain, and Marigold Extract.

7. A pet food formulation that is adapted for sporting dogs that comprises the following ingredients: Chicken, Ground Rice, Chicken Meal, Natural Flavor, Canola Oil Preserved with Mixed Tocopherols, Rosemary Extract and Citric Acid, Tomato Pomace, Brewers Dried Yeast, Chicory Root Extract, Potassium Chloride, Sodium Bicarbonate, Vitamins comprising Choline Chloride, Vitamin A Supplement, Vitamin D_3 Supplement, Vitamin E Supplement, Inositol, Niacin, Ascorbic Acid or a Source of Vitamin C, d-Calcium Pantothenate, Thiamine Mononitrate, Riboflavin Supplement, Beta Carotene, Pyridoxine Hydrochloride or Vitamin B_6, Folic Acid, Menadione Sodium Bisulfite Complex or a Source of Vitamin K activity, and Biotin, Vitamin B_{12} Supplement, Salmon Oil, Evening Primrose Oil, Minerals comprising Zinc Oxide, Zinc Proteinate, Ferrous Sulfate, Iron Proteinate, Manganous Oxide, Copper Sulfate, Copper Proteinate, Calcium Iodate, and Sodium Selenite, Glucosamine Hydrochloride, Hawthorn Berry Powder, Ginkgo Biloba Extract, *Yucca schidigera* Extract, Taurine, Panax Ginseng Root Powder, Chinese Ginseng Root, Spearmint Leaf Powder, Eyebright Powder, Siberian Ginseng Extract, Parsley Seed Oil Powder, Ginger Extract, Glutamine, and Marigold Extract.

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EXHIBIT E

US007037708B1

(12) **United States Patent**
Runge et al.

(10) **Patent No.: US 7,037,708 B1**
(45) **Date of Patent: May 2, 2006**

(54) **DRIED MICROORGANISM CULTURES AND METHOD FOR PRODUCING SAME**

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§ 371 (c)(1),
(2), (4) Date: **Oct. 11, 2000**

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C12N 1/20 (2006.01)

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(58) **Field of Classification Search** 435/252.1, 435/252.9, 243, 266; 424/93.4; 426/61, 471
See application file for complete search history.

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(74) *Attorney, Agent, or Firm*—Keil & Weinkauf

(57) **ABSTRACT**

Dry microorganism cultures comprising at least one microorganism species in carrier-bound form are present in the form of particles which a) have a particle size of at least about 0.1 mm and b) are compressed; processes for preparing dry microorganism cultures and their use for preparing foodstuffs and feedstuffs are also claimed.

20 Claims, 1 Drawing Sheet

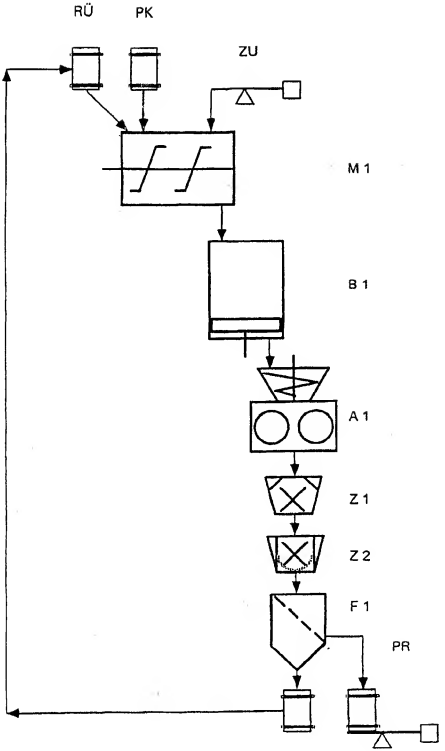

Fig.1

DRIED MICROORGANISM CULTURES AND METHOD FOR PRODUCING SAME

The present invention relates to novel dry microorganism cultures which can be used in particular to prepare foodstuffs and feedstuffs, and to processes for preparing dry microorganism cultures.

A main area of application of microorganisms, such as bacteria and yeasts, is preparing foodstuffs and feedstuffs. Thus, for example, lactic acid bacteria, such as those of the genus Streptococcus sp. or Lactobacillus sp. are used in the preparation of milk products, such as sour cream, buttermilk, yogurt, kefir, coumis, curd cheese and in the preparation of sauerdough and for preserving uncooked sausage, such as salami. Lactic acid bacteria, such as those of the genus Lactobacillus sp., for instance, are also used in the production of feeds., such as silage.

The microorganism preparations required for preparing foodstuffs and feedstuffs are usually used in the form of starter cultures. These are generally not freshly prepared liquid cultures, but either cultures usually frozen in liquid nitrogen or dry preparations. Dry preparations are usually preferred, since their transport and storage is technically less complex in comparison with frozen preparations.

Very varied types of dry preparations of microorganism cultures are known from the prior art. Thus, for example, EP-A-0 131 114 describes a Lactobacillus preparation where a bacterial cell suspension is applied to a pulverulent or granulated carrier composition and dried. However, to store the preparation it is necessary to package this in an oxygen-free protective gas atmosphere. DD 840493952 proposes freeze-drying strains of cultured microorganisms for producing starter cultures, packaging them in film and storing them at 279 to 288 Kelvin. JP-A-06/217713 describes the production of special Lactobacillus starter cultures by spray-drying. EP-A-0 202 409 proposes subjecting dry cultures to a wet granulation, processing the granules to form spherical particles and then drying them. In addition, proposals are made in a number of publications to provide coated dry bacterial preparations (cf. U.S. Pat. No. 3,677,897 for example).

A number of different processes are described in the prior art to produce dry microorganism preparations. In addition to the freeze-drying and fluidized-bed drying processes mentioned above, another alternative production method is spray-drying a microorganism suspension. Thus, for example, Stadhouders, J. et al., in Neth. Milk Dairy J. 23 (1969) 182 describes the spray-drying of lactic acid bacteria at 70° C., coupled with a post-drying step at 27° C. in vacuo. Apparently, preconditioned, i.e. predried, air is not used for the drying. Before the drying, a calcium hydroxide slurry is added to the material to be sprayed. The calcium lactate formed during the spray-drying is advantageous, inasmuch as it is said to have a lower hygroscopicity. In other spray-drying processes known from the prior art, bacterial suspensions to which the most varied types of carrier materials have been added in advance are sprayed. Thus, for example according to SU 724113, a bacterial suspension admixed with dried milk powder, molasses and sodium glutamate is sprayed. According to SU 1616990, a bacterial suspension admixed with the mineral palygorskite is spray-dried. WO-A-88/06181 describes the spray-drying of a bacterial suspension admixed with clay. JP-A-69/67989 describes the spray-drying of yeast cells or bacterial cells which are suspended in a neutral or slightly acidic solution which comprises proteins, carboxymethylcellulose, alginate or alginate ester, disaccharides or higher saccharides or polyhydric alcohols.

The dry microorganism preparations which are known to date from the prior art, in particular those preparations which are used for producing foodstuffs or feedstuffs, have at least one of the following disadvantages:

1) the content of viable microbes per unit weight of the dry material is very low owing to the production method, so that large volumes of the dry preparation must be used in the final application;
2) the storage stability is too low, so that the dry preparations must be used within a few weeks, if storage under technically complex conditions is impossible;
3) the dry preparations have a high dust content, which makes their processing more difficult;
4) the mechanical stability is very low, so that on mixing the preparation with mineral additives, a finely divided abraded material is formed and separation of the solid preparation can be observed;
5) the dissolution rate of the dry preparations is not satisfactory, so that the desired microbiological process for producing the foodstuff or feedstuff only begins slowly and unwanted microorganisms are given the possibility of multiplying, which can lead to considerable losses in quality.

The production processes known to date from the prior art, in particular the spray-drying processes described to date, are also unsatisfactory for at least one of the following reasons:

1) the processes are technically very complex;
2) the microorganism survival rate in drying is too low;
3) the moisture content of the dry product is too high.

A first object of the present invention is thus the provision of improved dry microorganism cultures which substantially no longer have the abovementioned deficiencies known from the prior art. In particular, starter cultures which are improved in comparison with the prior art are to be provided. The starter cultures according to the invention are especially to enable improved production of silage.

A second object of the present invention is the provision of improved processes for producing dry microorganism cultures. In particular, an improved process for spray-drying microorganism cultures should be provided which enables the production of dry preparations having a high content of viable microbes and high storage stability.

The above first object is achieved by providing a dry microorganism culture which comprises at least one microorganism species in carrier-bound form, wherein the culture is present in the form of particles which

a) have a particle size of at least about 0.1 mm and
b) are compressed.

The particulate cultures according to the invention are virtually dust free on account of the chosen particle size. The dust content is preferably in the range from about 0.01 to 0.05% by weight, based on the total weight of the dry culture. This corresponds to a dust index in the range from about 1 to 12 determined gravimetrically by a Casella instrument.

The particles according to the invention furthermore have a compressed, i.e. compact, structure. This is preferably achieved in their production by a compression step which is explained in more detail below and has not been previously described for dry microorganism preparations. In this operation a preliminary product obtained, for example, by spray-drying, freeze-drying or fluidized-bed-drying (such as the powder concentrate which is obtainable by a spray-drying variant according to the invention and is described below),

which usually has a significant dust content (e.g. a dust index from about 25 to 100), is mechanically compressed.

The compression can be performed, for example, by compacting the pulverulent preliminary product under the action of linear forces, e.g. in the range from about 5 to about 25 kN/cm, in particular from about 10 to about 15 kN/cm, in conventional compacting apparatuses, for example. However, the preliminary product can also be tabletted under the action of pressures in the range from about 50 to about 250 MPa, in particular in the range from about 80 to 200 MPa, such as from about 90 to about 160 MPa, for instance, in conventional tabletting presses, for example. Particular preference is given to compression by compacting. In addition, preference is given to compacting powder concentrates obtained according to the invention by spray-drying.

The provision of microorganism cultures of the type described above surprisingly results in the processing, in particular as starter cultures, being markedly simplified and, moreover, the mechanical stability of the particles and thus the danger of separation of starter culture preparations being markedly decreased. Surprisingly, it has also been found that the compression of the pulverulent preliminary product virtually does not impair product quality with respect to the number of viable microbes. Rather, owing to the high density achieved, the ingress of air and moisture into the dry preparations according to the invention is significantly decreased in such a manner that a considerable improvement in storage stability can be achieved.

Thus, for example, survival rates of 60% and above after storage for one year at room temperatures were achieved. Advantageous storage stability data of this type have not been described hitherto.

In particular, the compressed particles can comprise compacted broken material (i.e. material obtained by comminuting with or without classifying compacted product extrudates) having a diameter of from about 0.1 mm to about 2 mm, preferably from 0.3 to 1.25 mm. The diameter here is a value calculated from the total mass distribution of the compressed particles and corresponds to the diameter of spheres of equal mass. The edge length of the particles is in the range from about 0.1 to 2 mm, in particular from about 0.1 to 1.4 mm.

The compressed particles can, furthermore, be present as tablets of any desired shape, such as round, polygonal or oval, having a diameter of from about 2 to 50 mm and a ratio of diameter to thickness of from about 1:0.1 to about 10:1, in particular from about 1:1 to about 5:1.

According to a further preferred embodiment of the invention, the dry microorganism cultures comprise, as further component, an effervescence additive, comprising an acid component, such as an organic nonvolatile carboxylic acid, and a gas-forming component, such as a CO_2-forming component. Effervescence formulations of this type have the particular advantage of a surprisingly rapid dissolution after application of the starter culture. As a consequence of this rapid dissolution of the starter culture in its surrounding medium, rapid multiplication of the starter culture microorganisms is ensured, as a result of which losses in quality of the product to be produced using the starter culture can be avoided surprisingly well.

Preferably, the dry culture compressed according to the invention comprises, as carrier, at least one matrix material for embedding the microorganism cells with or without at least one other additive which stabilizes the cells.

The carrier used in the dry cultures according to the invention comprises at least one matrix component added as coformulant prior to the drying to usually freshly grown microorganisms, selected from mono-, oligo- and polysaccharides, polyols, polyethers, polymers, such as CMC or PVP, oligo- and polypeptides, from natural sources, such as milk, meat or cereals, derived substances or mixed substances, such as sweet whey powder, wheat semolina bran, peptone, alginates, mineral compounds, or mixtures of such matrix substances. In addition, additives having a stabilizing action can be added together with the matrix substance or later, for example antioxidants, such as α-tocopherol or ascorbic acid, or mixtures thereof. Furthermore, a stabilizing action can be exerted by other substances, which are selected from inorganic salts, such as alkali metal chlorides or alkaline earth metal chlorides, inorganic or organic buffers, such as alkali metal phosphate buffer, amino acids, such as aspartic acid or glutamic acid and the salts thereof, organic carboxylic acids, such as citric acid, organic nonvolatile solvents, such as DMSO, and other compounds, such as β-carotene and mixtures of these.

The microorganism cultures according to the invention preferably comprise viable microorganisms in a concentration of 10^8 to 10^{12} cfu (colony forming units)/g of dry culture. The powder concentrates produced according to the invention comprise from about $5 \cdot 10^8$ to $1 \cdot 10^{12}$, preferably about $4 \cdot 10^{11}$ to $8 \cdot 10^{11}$ cfu/g. The compressed cultures according to the invention comprise from about $1 \cdot 10^{11}$ to $4 \cdot 10^{11}$, in particular about $3 \cdot 10^{11}$ cfu/g. Starter cultures for producing silage comprise from about 1 to $7 \cdot 10^{10}$, in particular about $3 \cdot 10^{10}$ cfu/g.

In this process the microorganisms can be derived from one microorganism species or a plurality. A particularly preferred species are lactic-acid-producing bacteria, such as those which are suitable for silage production, such as, for example, Lactobacillus plantarum.

For the purposes of the invention, silage comprises feed plant products which have been preserved by the action of microorganisms, for example those based on grass, clover, straw, corn plants, fodder beets, legumes, cereals, such as corn and wheat, and the like.

The second object of the present invention described above is surprisingly achieved by providing a spray-drying process for producing a dry microorganism culture, comprising at least one microorganism species in carrier-bound form, which comprises

a) dissolving or suspending at least one substance suitable for forming a carrier in a liquid comprising at least one microorganism species,

b) drying the resultant mixture in a spray-dryer, for the spray-drying use being made of a conditioned dried gas heated to a temperature in the range of above about 80° C., in particular from about 90 to about 135° C., preferably from about 100 to about 110° C., such as about 105° C., and

c) removing the dried material from the spray-dryer, this dried material having an exit temperature of from about 40 to 85° C., in particular from about 45 to 75° C., preferably from about 50 to 65° C., such as about 55° C.

This spray-drying process according to the invention is also called carrier-bound spray-drying process below. The gas used for the drying is preferably a dried gas having a dew point of below +5° C. in particular having a dew point of from about −10 to about −50° C., such as conditioned compressed air or conditioned nitrogen. For example, compressed air having a dew point of about −25° C. and nitrogen having a dew point of about −40° C. can be used. A dew point of +5° C. is equivalent to roughly 5 g of water per m^3 of air.

According to a preferred embodiment of the spray-drying process according to the invention, in a downstream further stage d), the dried material is subjected to a post-drying. The post-drying temperature is in the range of from about 15 to 50° C., such as from about 25 to 40° C. The post-drying is performed, for example, in a gas atmosphere or in vacuo; alternatively to this, there is also the possibility of mixing a desiccant homogeneously with the dry microorganism preparation obtained in accordance with stage c).

Because of its design, the spray-drying process according to the invention surprisingly permits microorganism suspensions to be dried at survival rates of up to 100%. Owing to the use of conditioned gas in the spray-drying as well as the optional post-drying step, surprisingly, dry preparations having an extremely low moisture content (of from about 2 to 3% by weight of water), corresponding to a water activity a_w, of from 0.03 to 0.15, are provided. This directly causes the microorganism cultures which have been spray-dried according to the invention, with or without post-drying, to have survival rates of up to 60% after storage for 1 year at ambient temperature and ambient air conditions.

Owing to the surprisingly high survival rate in the above-described spray-drying, the content of viable microorganisms is markedly high. The resultant spray-dried product is therefore also called powder concentrate and, to reduce the concentration of viable cells, can be further diluted, depending on the field of application. The powder concentrate is particularly suitable for preparing the above-described compressed particulate cultures according to the invention.

The present invention therefore also relates to a process for producing the above-described compressed microorganism cultures, which comprises

i) producing a powder concentrate of the microorganism culture by carrier-bound spray-drying, carrier-bound freeze-drying or carrier-bound fluidized-bed drying,

ii) with or without admixing the powder concentrate with one or more coformulants and

iii) compressing this mixture by compacting or tabletting.

Preferably, in a further process step, the compressed mixture is broken, i.e. comminuted, and may be classified to give compressed granules of the desired size using a screen of suitable mesh width.

The present invention further relates to a process for producing a dry agglomerated microorganism culture, which comprises

i) producing a powder concentrate of the microorganism culture by carrier-bound spray-drying, carrier-bound freeze-drying or carrier-bound fluidized-bed drying,

ii) with or without admixing the powder concentrate with one or more coformulants and

iii) compressing this mixture by agglomeration.

Carrier-bound means here the presence of at least one matrix material of the above-described type during drying.

According to a preferred embodiment of the above-described compacting process or tabletting process or agglomeration process, stage i) is carried out in particular in accordance with the above-described spray-drying process.

The product obtained by the above-described compression processes is, for the purposes of the present invention, also called compressed or compacted dry concentrate (in the cfu range from about $1 \cdot 10^{10}$ to $1 \cdot 10^{11}$) and can be marketed as such, e.g. as a concentrated starter culture.

The present invention further relates to the use of the compressed dry microorganism cultures according to the invention as starter cultures for producing foodstuffs, such as for the production of milk products, such as sour cream, buttermilk, yogurt, kefir, coumis, curd cheese, for producing sourdough, uncooked sausage, and for producing feedstuffs, such as silage. For this purpose, the culture, with or without dissolution, is mixed with the foodstuff substrate or feedstuff substrate. If the cell count in the starter culture should be too high, it may be diluted, e.g. by mixing with an inert solid, such as lime, in particular feed lime.

The present invention further relates to foodstuffs and feedstuffs which have been produced using the starter cultures according to the invention.

The present invention is described in more detail in the sections now following with reference to the accompanying figure.

FIG. 1 shows diagrammatically a possible way of producing, from powder concentrate, granules compacted in accordance with the invention.

USABLE MICROORGANISMS

The present invention is not restricted in principle to certain microorganism cultures. Rather, those skilled in the art recognize that the present invention is applicable to any microorganisms, in particular bacteria and yeasts, which can be converted to a dry microorganism preparation under the conditions specified in the present description. A suitable group of microorganisms which can be used according to the invention are the group of lactic-acid-producing bacteria. In particular, these are bacteria which are suitable for the homofermentative lactic acid fermentation, i.e. break down glucose to lactate via the fructose bisphosphate pathway. Typical representatives of this group are bacteria of the genera Lactobacillus sp., Streptococcus sp. and Pediococcus sp. Concrete examples of lactobacilli which may be mentioned are *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bifidus, Lactobacillus casei, Lactobacillus lactis, Lactobacillus delbrueckii, Lactobacillus thermophilus, Lactobacillus fermentum, Lactobacillus brevis* and *Lactobacillus plantarum*. Examples of suitable streptococci are *Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetilactis, Streptococcus thermophilus, Streptococcus pyrogenes, Streptococcus salivarius, Streptococcus faecalis, Streptococcus faecium*; and examples of suitable pediococci are *Pediococcus cerevisiae* and *Pediococcus acidilactici*.

Fermentation of the Microorganisms

To carry out the present invention, preferably, use is made of freshly prepared microorganism suspensions. The fermentation media or fermentation conditions optimum for each microorganism are either known from the prior art or can be determined in only a few routine experiments by the person skilled in the art who is entrusted with the culture of microorganisms.

However, usually, the fermentation is carried out in such a manner that starting from a liquid or semi-solid preliminary culture (culture volume from about 10 to 200 ml), freshly prepared sterile fermentation medium is inoculated under sterile conditions, where the volumetric ratio of preliminary culture to culture medium can be from about 1:50 to 1:200. Preferably, freshly grown preliminary cultures are used which are in a late phase of logarithmic growth. Depending on the microorganism being grown, they are cultured under specific optimized growth conditions (such as temperature and pH). Usually, the growth temperature is in the range from about 20 to 40° C., but, for example when thermophilic bacteria are being grown, markedly higher temperatures can be present. The fermentation batch is kept

uniformly agitated, for example by moderate stirring or introducing air or nitrogen in order to prevent the development of temperature or substance gradients and to ensure continuous growth in this manner. After the growth phase is complete (determined for example by achieving a defined cell density or consumption of one of the added nutrients), the cell suspension can be used directly to produce the dry preparations according to the invention.

However, it is also possible to concentrate the resultant original cell suspension to increase the cell count. Suitable methods for this are, for example, centrifugation, ultrafiltration or thin-film evaporation. However, a centrifugation step is usually used to concentrate the cell suspension, which centrifugation step is preferably carried out at a decreased temperature, that is to say in the range from about 4 to 10° C.

Instead of the concentration, or in combination with it, there is also the possibility of subjecting the freshly cultured cell suspension to a washing step in order to remove culture constituents, such as metabolic products, which may have an adverse effect on the activity. In this case, the procedure usually adopted is that, preferably at from about 4 to 10° C., the original culture broth is first concentrated to give a suspension of high cell density and this is then taken up in a suitable buffer solution and diluted to the desired cell density. If necessary, the washing step can also be repeated a plurality of times. Solids contents which can be used according to the invention of cultures of microorganisms suitable for producing the dry preparations according to the invention are usually in the range from about 5 to 25% by weight, such as from about 10 to 20% by weight.

The microorganisms can be cultured by batch fermentation or continuously.

To further illustrate the invention, in the section below, a more detailed description is given of culturing a lactic acid bacterium, in particular Lactobacillus plantarum. This is a bacterium which is to be found in particular on intact and decomposing plants and is particularly suitable for producing silage feedstuffs.

A suitable fermentation medium comprises, per liter of medium, from about 40 to 60 g of glucose, from about 30 to 60 g of yeast extract and a cocktail of customary trace elements, such as magnesium, manganese and, optionally, iron. The pH of the fermentation medium is from about 6 to 7. The fermentation temperature is from about 33 to 38° C. The pH of the fermentation medium can be kept within the desired range by adding sterile sodium hydroxide solution. Growth is complete when glucose consumption or lactic acid synthesis can no longer be observed.

According to a lactobacillus fermentation variant which is suitable according to the invention, after about 80% or 90% of the growth is achieved, the fermentation medium temperature is increased to from about 42 to 46° C. until the added glucose is completely consumed. It has been found according to the invention that cultures produced in this manner are particularly stable in particular in the spray-drying, as a result of which high survival rates are achievable. Comparable growth variants are also conceivable with other microorganisms which can be used according to the invention.

After growth is complete, the fermentation batch is brought to the desired cell density. If desired, the cell suspension can be washed until it is virtually lactate free. The cell count of a microorganism suspension suitable according to the invention is usually in the range from about 1×10^{10} to about 5×10^{12} cfu/g of suspension.

Carrier Substances

The dry microorganism cultures prepared according to the invention, in addition to any nonvolatile constituents present from the respective fermentation batch, such as metabolic products, comprise at least one matrix material with or without other stabilizing substances. These coformulants are preferably selected from inorganic salts or buffers, at least one other compound which is selected from mono-, oligo- and polysaccharides, polyols, polyethers, amino acids, oligo- and polypeptides, milk-derived compounds, organic carboxylic acids, mineral compounds, organic carrier materials such as wheat semolina bran, alginates, DMSO, PVP (polyvinylpyrrolidone), CMC (carboxymethylcellulose), α-tocopherol, β-carotene and mixtures thereof.

Examples of suitable saccharide carrier components are sucrose, fructose, maltose, dextrose, lactose and maltodextrin. An example of a suitable polyol is glycerol. Examples of suitable amino acids are glutamic acid, aspartic acid and the salts thereof. An example of a suitable peptide carrier is peptone. An example of a milk-derived compound is, in addition to the abovementioned maltodextrin, also sweet whey powder. Suitable organic carboxylic acids are, for example, citric acid, malic acid and L-ascorbic acid. Examples of suitable mineral carriers are montmorillonite and palygorskite.

However, preferably, as carrier for the dry microorganism preparations according to the invention, use is made of mixtures of the abovementioned classes of substances. Mixtures of this type preferably comprise, as main component, a matrix material, such as one of the abovementioned saccharide components or, for example, sweet whey powder, with or without a minor content of at least one further component, such as a buffer component (for example citric acid) or an antioxidant (for example L-ascorbic acid or α-tocopherol). The addition of further stabilizing constituents, such as sodium glutamate and/or peptone, has likewise proved to be advantageous.

The matrix component is customarily used in carrier compositions usable according to the invention in about 5 to 30 times the amount of the other carrier constituents. Examples of particularly suitable carrier combinations are:

a) sweet whey powder/citric acid/L-ascorbic acid (weight ratio about 40:1:1).

b) maltodextrin/lactose/citric acid/L-ascorbic acid (weight ratio about 20:20:1:1), unsupplemented or supplemented by about 1.5 parts of β-carotene and 0.5 part of α-tocopherol per part of citric acid.

c) maltodextrin/sodium glutamate/L-ascorbic acid (weight ratio about 10:1.5:1).

d) lactose/glucose/peptone/citric acid (weight ratio about 6:6:1.2:1).

The carrier substances according to the invention can be added to the microorganism suspension either as solid or in dissolved form. However, preferably, a sterile solution of the carrier/carriers is prepared, this is cooled to a temperature of from 4 to 10° C. and this is mixed with the likewise cooled microorganism suspension with gentle stirring. To prepare a homogeneous suspension, the resultant mixture is stirred with further cooling for a period of from about 10 minutes to 1 hour.

Preparation of Dry Microorganism Preparations

The microorganism suspension containing the carrier added in the manner described above can then be dried in various ways. Suitable drying processes are in principle

freeze drying, fluidized-bed drying and, preferably, spray-drying. For the purposes of the present invention, spray-drying also comprises modified spray-drying processes, such as spray-agglomeration or agglomerating spray-drying. The latter process is also known under the name FSD (fluidized spray-dryer) process.

Freeze-drying for preparing dry microorganism cultures according to the invention can be carried out, for example, on the basis of the freeze-drying process described in EP-A-0 259 739 or U.S. Pat. No. 3,897,307. The contents of these publications are hereby incorporated completely by reference.

A suitable fluidized-bed drying process is described, for example, in the dissertation by U. Kessler on the subject "Experimentelle Untersuchung und Modellierung der Überlebensrate von Milchsäurebakterien bei der thermischen Trocknung" [Experimental study and modeling of the survival rate of lactic acid bacteria during thermal drying], Technical University of Munich, 1993. The contents of this publication are likewise incorporated completely by reference. To carry out the fluidized-bed drying process, it is advantageous that the carrier material to be used, in particular the matrix component, is introduced in a fluidized bed and this is sprayed with the microorganism suspension in the manner described by U. Kessler.

However, the drying process which is most preferred according to the invention is spray-drying. Those methods which can be used according to the invention are essentially all spray-drying techniques known hitherto. The material to be sprayed can, for example, be dried cocurrently or countercurrently; spraying can be carried out by means of a single-component or multiple-component nozzle or by means of an atomizer wheel.

Preference is given according to the invention to the use of material to be sprayed having a solids content (after addition of the carrier) of from about 10 to 40, such as from about 10 to 25% by weight.

The spray-drying process according to the invention is carried out in such a manner that a conditioned dry gas having a temperature in the range of above about 80° C. is introduced into the drying apparatus. In particular, the inlet temperature should be in the range of from about 90 to 135° C. Particular preference is given to a drying temperature in the range of about 105° C. The rate of the drying process is designed according to the invention in such a manner that the exit temperature of the drying material from the dryer is in the range of about 45 to 75° C., in particular in the range of from about 50 to 65° C., preferably about 55° C.

Of particular importance to the process according to the invention is the use of preconditioned, i.e. low-moisture, drying air. Preferably, use is made of compressed air having a dew point at about −25° C.

The drying process according to the invention shall be carried out in such a manner that a very low residual moisture content is present in the dry material. Preferably, the water activity a_w in the drying material should be less than 0.4. However, to further improve the long-term storage stability, according to the invention water activities of less than 0.15, preferably in the range from about 0.03 to 0.1 are sought after. The percentage water content is preferably from about 2 to 3% by weight. Most preferably, this is achieved by adding a post-drying step subsequently to the spray-drying step. The drying material for this purpose is, for example, post-dried in a fluidized bed, preferably at a temperature in the range of from 15 to 50° C., for a period of, for example, from 15 minutes to 20 hours. Again, preferably, conditioned compressed air or conditioned nitrogen serves as drying gas. However, the post-drying can also be performed by applying a vacuum of from about 1 to 50 mm Hg for a period of from about 15 minutes to 20 hours and at a temperature of from about 15 to 50° C. In this case, preference is given to stirring the drying material, for example, using a paddle agitator.

Instead of the above-described physical post-drying processes, it is also conceivable to add specific desiccants to the dry material obtained from the spray-drying. Desiccants of this type should themselves have a very low water activity, such as an a_w of 0.01 or less. Examples of suitable desiccants are inorganic salts, such as calcium chloride and sodium carbonate, organic polymers, such as the product obtainable under the trade name Kollidion 90 F, and silicon-dioxide-containing desiccants, such as silica gel, zeolites and desiccants which are obtainable under the trade name Tixosil 38, Sipernat 22 S or Aerosil 200.

According to the invention, it was surprisingly found that, despite the relatively high drying temperatures, the survival rate for the dry preparations according to the invention had excellent values, namely of 75%±25%.

The content of viable microorganisms is in the range of from about 5×10^8 to 1×10^{12} cfu/g of dry matter. These preparations are also called according to the invention powder concentrates. Since, for individual final applications, lower contents of viable microorganisms are also completely sufficient, powder concentrates of this type can therefore if appropriate be blended to the final count of viable microorganisms by mixing with further inert carrier material.

Preparation of Compressed Dry Microorganism Cultures

The powder concentrates obtainable by the above-described drying processes usually have a relatively high dust content and are thus not yet satisfactorily handleable for individual applications. Furthermore, various applications require an increased mechanical stability of the dry cultures. It is therefore necessary to improve the properties of the above-described powder concentrates by a further compression step.

To reduce the dust content of the powder concentrates according to the invention, it is possible to agglomerate these in a conventional manner to form granules, or using external forces, to compact them or tablet them.

Agglomeration is a generally known process, and is described, for example, by Schade, A. and Leuenberger, H. in Continuous fluidized-bed spray granulation, Chemie Ingenieur Technik (1992) 64 (1992) 1016; Uhlemann, H., Preparation of pharmaceutical granules in a combined wet granulation and multichamber fluidized-bed drying process, Chemie Ingenieur Technik 62 (1990), 822; or Rosch, M. and Probst R., Granulation in the fluidized bed, Verfahrenstechnik (1975), 9, 59.

Use can be made according to the invention of agglomeration using a mixer. For this purpose, the above-described powder concentrate is charged into the mixer and oil, water or an aqueous or alcoholic solution of sugars, polymers or other additives is sprayed in to agglomerate the powder concentrate.

In addition, use can be made according to the invention of agglomeration in a fluidized bed. In this case, powder concentrate is vortexed with gas feed and sprayed with an aqueous or alcoholic solution of sugars, polymers or other additives to form the agglomerate. Suitable processes for this purpose are described, for example, in WO-A-88/06181,

in the dissertation by U. Kessler (loc. cit.) and by K. Fuchs in ZFL 45 (1994) 31. The disclosure of the abovementioned publications is hereby incorporated by reference.

Agglomeration produces granulated microorganism cultures having a particle size in the range of from about 0.1 to about 4 mm, in particular from about 0.3 to 2.5 mm.

However, particularly preferred according to the invention is the preparation of dry microorganism cultures which are present in the form of particularly highly compressed particles. This is carried out according to the invention either by tabletting in conventional tablet presses or with the use of conventional compacting apparatuses equipped with two counter-rotating rolls.

To compress the powder concentrates obtainable according to the invention, to these are usually added one or more coformulants or additives to modify the processability to the end product or the properties of the end product.

To improve the flowability of the powder concentrate, a free-flowing agent is preferably added. Examples of a suitable free-flowing agent are spray-dried silicon dioxide powders, which are obtainable, for example, under the trade name Sipernat 50. To improve the storage stability of the solid formulations according to the invention, in addition, conventional antioxidants, such as L-ascorbic acid, can be added. Furthermore, desiccants of the above-described type can additionally be added.

The action of the cultures according to the invention is markedly improved if measures are taken which, after the culture has been applied, lead to a rapid breakdown of the grain structure and thus to a rapid release of the microorganisms. One possibility of achieving this is the addition of a readily water-soluble component which thus accelerates the breakdown of the grain structure. Suitable compounds are, for example, poly(ethylene glycol)s, which are obtainable, for example, under the trade name Pluriol E.

Another solid formulation particularly preferred according to the invention comprises what is termed an effervescence additive. This is a gas-releasing component, in particular a CO_2 source, for example an alkaline earth metal hydrogen carbonate, preferably sodium hydrogen carbonate or ammonium hydrogen carbonate, and an acid component, preferably selected from citric acid, ascorbic acid or malic acid. This effervescence additive, in the presence of moisture, produces a spontaneous gas formation with breakdown of the grain structure and rapid release of the microbial cells.

In particular, to prepare highly compressed, compacted or tabletted microorganism cultures, it is advisable to add compacting or tabletting aids. This is because it has surprisingly been found according to the invention that adding such compacting aids decreases the pressures acting on the microorganisms during the compacting and thus markedly improves the survival rate of the microbes. Examples of suitable compacting aids are microcrystalline cellulose, sugars and mixtures thereof. Concrete examples of microcrystalline cellulose are products which are commercially available under the trade names Avicel, Arbocel and Vivapur. Examples of suitable sugars are maltose, maltodextrin and lactose preparations, which are obtainable under the trade names Granulac, Tablettose or FloLac. An example of a suitable mixed cellulose/sugar product is the commercial preparation Cellactose. A further suitable tabletting aid is a lactose preparation granulated using PVP, obtainable under the trade name Ludipreß.

Other suitable additives are poly(ethylene glycol)s (Mw from 100 to 10,000) which can have a stabilizing action on the cells embedded in the matrix.

The accompanying FIG. 1 shows a flow diagram for the further processing according to the invention of the powder concentrates to give a compacted product according to the invention. Powder concentrate PK is mixed in the mixer M1 with the coformulants or additives ZU, passes from there into a reservoir B1 which feeds the compactor A1. The product ribbon exiting from the compactor is precommunuted or finely comminuted in the grinders Z1 and Z2 and in the screen F1, product PR is separated off from dust fractions having a particle size of less than 0.3 mm. This material is fed to the mixer M1 as recycled material RÜ. The product PR having a particle size of 0.3 mm or above, such as from 0.3 to 1.5 mm, passes to the packaging station or may be subjected to further processing, such as a coating process.

Suitable coating materials, which preferably are additionally to hinder the ingress of moisture to the dry preparation, are, for example, alcoholic solutions of PVP, in particular a PVP product which is commercially available under the trade name Kollidon VA64. Another usable coating system is a mixture of shellac and Kollidon 25 or 30, which is supplemented with titanium dioxide and tallow and is likewise present in alcoholic solution.

To reduce the cell count further if necessary, a coated or uncoated product obtained in this manner can be blended, for example, with lime, or another suitable mineral additive.

EXAMPLES

Analytical Methods Used in the Following Examples

a) Cell Count Determination:

Cell counts are determined in the conventional manner by serial dilution with sterile 0.9% strength NaCl solution and subsequent plating on MRS agar (Difco Laboratories). Colony-forming units (cfu) were counted after incubation for 48 hours at 37° C. Only plates which contained between 30 and at most 300 colonies were evaluated. Generally, 3 plates per stage were evaluated and the mean taken.

The specific cell count of a sample was determined by calculation, dividing the cell count per gram of sample by the relative sample dry matter content.

b) Determination of the Survival Rate on Drying:

The survival rate during drying was calculated from the specific cell count of the sample before drying divided by the specific cell count after drying. It was always expressed in percent.

c) Determination of Storage Stability:

To determine the storage stability of a dried sample, the specific cell count of the dried sample was determined immediately after drying (day_O). The dried cell material was stored under air in an opaque tightly sealed vessel at room temperature (21° C.±2° C.) for extended periods. The specific cell count was determined again at regular intervals (day_N). The storage stability was calculated from the quotient of specific cell count day_N/specific cell count day_O.

If the specific cell count after drying was, for example, $5 \cdot 10^{11}$ cfu/g of DM and, after storage for 8 weeks, $4 \cdot 10^{11}$ cfu/g of DM, the storage stability was 80% of the initial value.

d) Moisture Content Measurement:

Electronic moisture analyzer HR 73 from Mettler Procedure: approximately 2 g of powder are distributed onto the measuring scales of the instrument. Measurements are taken at a drying temperature of 105° C. up to constant weight (switch-off criterion: max. 1 mg of weight loss in 50 seconds).

e) Measurement of Water Activity:

Hygroscope DT instrument from Rotronic AG, Zürich, Switzerland The product is placed in the sample holder and this is positioned in the measuring chamber thermostated to 25° C. After closing the measuring chamber and an equilibration time of 20 minutes, the instrument measurement value is read off.

f) DSC Measurement to Determine the Glass Transition Temperature T_g:

TA4000 instrument from Mettler Sample weight approximately 15 mg, heating rate 20° C./min, samples were flushed with a nitrogen stream during measurement.

DSC=Differential Scanning Calorimetry

Microorganism Culture Examples

Example K1

Batch Fermentation 10 Liter Scale

10 l of a fermentation medium which comprised the following constituents were placed in a 14 l fermenter and sterilized at 121° C. for 30 minutes:

Glucose monohydrate	550.0 g
50% yeast extract suspension (pH 4.5 with phosphoric acid)	750.0 g
Tween 80 ®	10.0 g
$MgSO_4 * 7 H_2O$	5.0 g
$MnSO_4 * 1 H_2O$	0.5 g

After sterilization, the medium was adjusted to pH 5.8 at 37° C. using sterile 25% strength sodium hydroxide solution and the medium was blanketed with a gentle stream of sterile nitrogen. The fermenter was stirred at 150 rpm.

The fermenter was then inoculated with 100 ml of a preculture of Lactobacillus plantarum (BASF strain LU 3244) which had previously been grown for 16 h at 37° C. in MRS nutrient medium (Difco Laboratories). The culture pH was continuously kept at 6.2 using 25% strength sodium hydroxide solution.

The course of the fermentation was followed from the sodium hydroxide solution consumption. As soon as no more sodium hydroxide solution was consumed (total consumption 890 g), all of the fermentation broth was drained off and centrifuged at 8° C. The biomass was resuspended in about 600 g of supernatant and made up to exactly 1000 g with supernatant. The dry matter content was determined using an infrared drying balance (105° C. to constant weight). The solids content of this suspension was 15%.

Example K2

Batch Fermentation 200 Liter Scale

180l of a fermentation medium which comprised the following constituents were placed in a 200 l fermenter and sterilized at 121° C. for 30 minutes:

Glucose monohydrate	11 kg
50% yeast extract suspension	15 kg
Tween 80 ®	200 g
$MgSO_4 * 7 H_2O$	200 g
$MnSO_4 * 1 H_2O$	10 g

After sterilization, the medium was adjusted to pH 5.8 at 37° C. using sterile 25% strength sodium hydroxide solution and the medium was blanketed with a gentle stream of sterile nitrogen.

The fermenter was then inoculated with 2000 ml of a preculture of Lactobacillus plantarum (3244) which had previously been grown for 24 h at 30° C. in MRS nutrient medium. The pH of the culture was continuously controlled using 25% strength sodium hydroxide solution.

The course of the fermentation was followed from the sodium hydroxide solution consumption. In total, 16.43 kg of 25% strength NaOH were consumed. As soon as sodium hydroxide solution was no longer consumed, all of the fermentation broth was drained off and harvested at 8° C. using a continuous separator. The harvested biomass had a weight after centrifugation of 20 kg, and the solids content of this suspension was 12.3%. The cell count of the suspension was $1.04 \cdot 10^{11}$ cfu/g of suspension. The specific cell count was $8.45 \cdot 10^{11}$ cfu/g of dry matter (DM).

Example K3

Batch Fermentation With Temperature Shock

A fermentation was carried out in a similar manner to Example 2. At a sodium hydroxide consumption corresponding to 90% of the expected value, the fermenter temperature was increased to 44° C. and kept until all of the sugar present had been consumed. The cells were then harvested as described in Example K2. The cell count of the fermentation broth was $1.8 \cdot 10^{11}$ cfu/g at a solids content of 21.17%. This corresponds to a specific cell count of $8.5 \cdot 10^{11}$ cfu/g DM.

Example K4

Continuous Fermentation

10 l of a fermentation medium having the following composition were charged into a 14 l fermenter and sterilized at 121° C. for 30 minutes (production fermenter):

Glucose monohydrate	400.0 g
50% yeast extract suspension (pH 4.5 with phosphoric acid)	500.0 g
KH_2PO_4	30.0 g
Citric acid monohydrate	21.0 g
Tween 80 ®	10.0 g
$MgSO_4 * 1 H_2O$	5.0 g
$MnSO_4 * 1 H_2O$	1.7 g
$(NH_4)_2Fe(SO_4)_2 * 6H_2O$	0.4 g

2000 l of the same medium were charged into a second fermenter having a total volume of 3000 and sterilized (reservoir fermenter). Both fermenters were connected by a sterilizable line. Via an intermediate vessel which stood on a balance, using an automatic control system, 3 l of fresh medium were pumped every hour into the production fermenter. The temperature of the production fermenter was controlled to 37° C. The pH was controlled to 5.8 using 25% strength NaOH. The fermenter was stirred at 150 rpm and blanketed with nitrogen at 0.1 VVM.

Via a second pump, 3 l of medium were continuously taken off every hour and collected in a stainless steel collection vessel precooled to from 0 to 4° C. The biomass concentration was determined by turbidimetry and was 3.5 g/l. The glucose, concentration in the production fermenter effluent was, after the initial growth phase, 0 g/l at all times. The cell count of the fermentation broth was $1.48 \cdot 10^{10}$ cfu/g of broth. The dry matter content of the fermentation broth was 6.89%, equivalent to 217 g DM. The specific cell count was $2.15 \cdot 10^{11}$ cfu/g of DM.

Example K5

Cell Harvest With Washing Step to Remove Sodium Lactate

72 l of fermenter discharge from Example K4 were harvested continuously at 8° C. using a commercial separator. About 7 kg of cell suspension was obtained. To this was added a washing solution which comprised 40 l of deionized water, 450 g of NaCl and 136 g of KH_2PO_4. The pH of the washing solution had been adjusted in advance to 7.0 using 25% strength sodium hydroxide solution. The about 50 l of resuspended cells were again separated. 3160 g of concentrated washed cell suspension were obtained. The solids content of the suspension was 9.97%. The cell count was $2.49 \cdot 10^{11}$ cfu/g of suspension. The specific cell count was $2.5 \cdot 10^{12}$ cfu/g DM.

This washed cell suspension was virtually free of sodium lactate. The biomass concentration was determined by turbidimetry to be 80 g/l.

Examples of Preparation by Spray-Drying of Powder Concentrates According to the Invention

The spray-drying experiments described in the following section for preparing powder concentrates according to the invention are carried out in a laboratory spray-dryer of type Niro Minor from Niro, Copenhagen, Denmark. The ready-to-spray bacterial suspension is sprayed via a two-component nozzle cocurrently with preconditioned heated compressed air into the plant drying tower, the dried product is separated from the air using a cyclone and collected.

Example S1

To prepare a coformulant solution, 200 ml of deionized water (completely demineralized water) are heated to 60° C. 150 g of sweet whey powder, 7.5 of NaCl, 3.8 g of KH_2PO_4, 3.8 g of citric acid and 3.8 g of L-ascorbic acid are dissolved therein, adjusted to pH 7 using 40% strength aqueous NaOH and made up to 400 g total mass using deionized water. This solution is cooled to 5° C.

200 ml of washed, i.e. essentially sodium-lactate-free centrifuged ferment (prepared in a similar manner to Example K5) (12.7% solids content (S.C.)) are placed in an ice bath at a temperature of 5° C. and 400 g of coformulant solution, cooled to 5° C., are added with stirring. The mixture of centrifuged ferment and coformulants is further stirred for 30 minutes at 500 rpm using a magnetic stirrer with ice bath cooling. By means of spray-drying (Niro Minor apparatus) the mixture is then converted into a powder concentrate A, which is separated off in the cyclone. In the course of this, the reservoir from which the mixture is metered is cooled to 4° C., the inlet temperature is from 105 to 110° C., the exit temperature is from 53.5 to 55.5° C. A two-component nozzle is used, conditioned air (dew point −25° C.) at 4 bar being used to spray the mixture of centrifuged ferment and coformulants.

The powder concentrate A is further dried at room temperature for 2 hours in a nitrogen-operated (dew point=−40° C.) fluidized bed, powder concentrate B being obtained.

Characterizations:

- Ready-to-spray mixture: 35% S.C., $2.84 \cdot 10^{11}$ cfu/g of dry matter
- Powder concentrate A: water activity a_w=0.135
- Powder concentrate B: water activity a_w=0.076, moisture content 3.4%, T_g from DSC measurement: 54° C., $1.98 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 70% survival rate in the drying)
- Storage study of powder concentrate B: cfu counts with room-temperature storage in containers sealed under ambient air: $2.0 \cdot 10^{11}$ cfu/g of dry matter (100%) after 30 days

Example S2

To prepare a coformulant solution, 200 ml of deionized water are heated to 70° C. 75 g of maltodextrin (Glucidex IT6, Roquette), 75 g of lactose, 7.5 g of NaCl, 3.8 g of KH_2PO_4, 3.8 g of citric acid and 3.8 g of L-ascorbic acid are dissolved therein, the mixture is adjusted to pH 7 using 40% strength aqueous NaOH and made up to 400 g total mass using deionized water. This solution is cooled to 5° C.

200 ml of washed, i.e. essentially sodium-lactate-free centrifuged ferment (16.5% S.C.; prepared similarly to example K5) are mixed, at 5° C., with stirring into 400 g of coformulant solution, cooled to 5° C. The mixture is stirred for 30 minutes at 250 rpm by a magnetic stirrer with ice bath cooling. 101 ml of a solubilized mixture prepared in accordance with EP-A-0 479 066 (BASF) from 25% Tween 80, 5% β-carotene and 2% α-tocopherol are then added and further stirred for 10 minutes with ice bath cooling. This mixture is then converted by spray-drying, as described in Example S1, into a powder concentrate A (inlet temperature 105° C., exit temperature from 54 to 55° C.). The powder concentrate A is not further dried.

Characterizations:

- Ready-to-spray mixture: 29% S.C., $3.84 \cdot 10^{11}$ cfu/g of dry matter
- Powder concentrate A: water activity a_w=0.065, moisture content 2.8%, T_g from DSC mensurement: 61° C., $2.22 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 58% survival rate in the drying)
- Storage Study on Powder concentrate A: cfu counts for room-temperature storage in containers sealed under ambient air: $1.9 \cdot 10^{11}$ cfu/g of dry matter (86%) after 30 days

Example S3

400 ml of unwashed, i.e. sodium-lactate-containing, centrifuged ferment (prepared similarly to Example K4)(14.3% S.C.) are placed in an ice bath at a temperature of 5° C. 57.2 g of Glucidex IT6, 8.6 g of L-ascorbic acid and 5.7% of sodium glutamate are stirred as solids into the cooled centrifuged ferment with stirring at 700 rpm by means of a magnetic stirrer. The pH is adjusted to 7 using 40% strength aqueous NaOH. The mixture of centrifuged ferment and coformulants is further stirred for 30 minutes at 500 rpm using a magnetic stirrer at approximately 3° C. with ice bath cooling. The mixture is then converted by spray-drying, as described in Example S1, into a powder concentrate A (inlet temperature 105° C.; exit temperature from 54.5 to 55.5° C.).

The powder concentrate A is further dried at room temperature in a nitrogen-operated fluidized bed for 2 hours, a powder concentrate B being obtained.

Characterizations:

- Ready-to-spray mixture: 27% S.C., $4.65 \cdot 10^{11}$ cfu/g of dry matter
- Powder concentrate A: water activity a_w=0.197
- Powder concentrate B: water activity a_w=0.072, moisture content 3.8%,

T_g from DSC measurement: 52° C.,
$4.64 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 100% survival rate in the drying)
Storage Study on Powder Concentrate B:
cfu counts with room-temperature storage in containers sealed under ambient air:
45 $4.1 \cdot 10^{11}$ cfu/g of dry matter (88%) after 28 days

Example S4

215 ml of washed, i.e. essentially sodium-lactate-free, centrifuged ferment (prepared similarly to Example K5) (14.5% S.C.) are placed in an ice bath at a temperature of 5° C. 31.2 g of Ghucidex IT6, 4.7 g of ascorbic acid and 3.1% of sodium glutamate are then stirred in as solids into the cooled centrifuged ferment with stirring at 700 rpm by a magnetic stirrer. The pH is adjusted to 7 using 40% strength aqueous NaOH. The mixture of centrifuged ferment and coformulants is further stirred for 30 minutes at 500 rpm using a magnetic stirrer with ice bath cooling. The mixture is then converted by spray-drying, as described in Example S1, into a powder concentrate A (inlet temperature 105° C.; exit temperature from 54.5 to 55.5° C.).

The powder concentrate is further dried at room temperature in a nitrogen-operated fluidized bed for 2 hours, powder concentrate B being obtained.
Characterizations:
Ready-to-spray mixture: 28% S.C., $8.76 \cdot 10^{11}$ cfu/g of dry matter
Powder concentrate B: water activity a_w=0.044, moisture content 3.8%,
T_g from DSC measurement: 48° C.,
$7.17 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 82% survival rate in the drying)
Storage Study on Powder
concentrate B: cfu counts for room-temperature storage in containers sealed under ambient air:
$3.7 \cdot 10^{11}$ cfu/g of dry matter (52%) after 27 days

Example S5

To prepare a coformulant solution 1, 40 ml of deionized water are charged and 33.3 g of lactose and 6.3 g of peptone are dissolved therein, the mixture is made up to a total mass of 83 g with deionized water and adjusted to pH 7 using 40% strength aqueous NaOH. To prepare a coformulant solution 2, 40 ml of deionized water are charged and 33.3 g of glucose-1-hydrate and 5.4 g of citric acid are dissolved therein, the mixture is made up to a total mass of 83 g with deionized water and adjusted to pH 7 using 40% strength aqueous NaOH. These solutions 1 and 2 are cooled to 5° C.

200 ml of washed, i.e. essentially sodium-lactate-free, centrifuged ferment (prepared similarly to Example K5) (12.7% S.C.) are mixed with 83 g of the cooled coformulant solution 1 in an ice bath at approximately 4° C. The mixture is stirred for 30 minutes with ice bath cooling. 83 g of the cooled coformulant solution 2 are then added with stirring and further stirred for 30 minutes with ice bath cooling. Then this mixture is converted by spray-drying, as described in Example S1, into a powder concentrate A (inlet temperature 105° C.; exit temperature 55° C.).

The powder concentrate A is further dried at room temperature for 2 hours in a nitrogen-operated fluidized bed, powder concentrate B being obtained.
Characterizations:
Ready-to-spray mixture: 29% S.C., $7.30 \cdot 10^{11}$ cfu/g of dry matter
Powder concentrate B: water activity a_w=0.139, moisture content 3.7%,
T_g from DSC measurement: 45° C.,
$5.06 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 69% survival rate in the drying)
Storage Study on Powder
concentrate B: cfu counts at room-temperature storage in containers sealed under ambient air:
$4.8 \cdot 10^{11}$ cfu/g of dry matter (95%) after 21 days

Example S6

The ready-to-spray mixtures were prepared in a similar manner to Example S3. Here, however, two different centrifuged ferments were used:

Example S6a: batch fermentation, with the ferment having been cooled to 4° C. for 40 minutes toward the end of the fermentation.

The powder concentrate A obtained in the spray-drying in accordance with Example S1 (inlet temperature from 107 to 111° C.; exit temperature from 58 to 61° C.) was not further dried.
Characterizations:
Ready-to-spray mixture: $3.68 \cdot 10^{11}$ cfu/g of dry matter
Powder concentrate A: $0.76 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 21% survival rate in the drying)

Example S6b: batch fermentation, with the ferment having been heated to 44° C. toward the end of the fermentation. In this example, the ready-to-spray mixture was divided. In a first experiment, the reservoir vessel was thermostatted to 4° C., as in Examples S1 to S5 and S6a. In a second experiment, the reservoir vessel was thermostatted to 20° C.

The powder concentrates A obtained by spray drying in accordance with Example S1 (inlet temperature from 103 to 110° C.; exit temperature from 59 to 61° C.) were not post-dried.
Characterizations for Rreservoir at 4° C.:
Ready-to-spray mixture: $3.53 \cdot 10^{11}$ cfu/g of dry matter
Powder concentrate A: $2.36 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 67% survival rate in the drying)
Characterizations for Reservoir at 20° C.:
Ready-to-spray mixtures:$3.53 \cdot 10^{11}$ cfu/g of dry matter
Powder concentrate A: $1.48 \cdot 10^{11}$ cfu/g of dry matter (equivalent to 42% survival rate in the drying)

Formulation Examples

In accordance with the formulas stated below, dry mixtures of powder concentrates according to the invention were prepared and processed to form compacted starter culture preparations:

Unless specified otherwise, the release agent used was Leucine and the free-flowing agent used was Sipernat 50S (spray-dried silicon dioxide).

The individual components of the preparations are first mixed with one another. For this purpose, for example, a plowshare mixer is used (type LÖ 20 from Lödige). The dry mixture obtained in this manner is compacted in a compactor. For example, for this purpose a laboratory compactor can be used which applies a pressing force of 14 kN/cm² (e.g. laboratory compactor L 200 from Bepex). The product ribbon exiting from the compactor is then comminuted to a particle size of ≦1.25 mm. The crude granules are screened to separate off fines of a particle size of ≦0.3 mm. The yield of useful material is about from 50 to 60% of the material used.

Example F1

Preparing a Compacted Effervescent Product for Use as Starter Culture for Silage

Preparation A:	
Powder concentrate (in accordance with Example S2)	200.0 g
Citric acid, anhydrous	95.0 g
$NaHCO_3$	95.0 g
PEG (M_w < 400)	8.0 g
Free-flowing agent	2.0 g
Preparation B:	
Powder concentrate (according to Example S2)	100.0 g
Ascorbic acid, powder	47.5 g
$NaHCO_3$	47.5 g
PEG (M_w < 400)	4.0 g
Free-flowing agent	1.0 g
Preparation C:	
Powder concentrate (according to Example S2)	100.0 g
Malic acid	47.5 g
$NaHCO_3$	47.5 g
PEG (M_w < 400)	4.0 g
Free-flowing agent	1.0 g
Preparation D:	
Powder concentrate (in accordance with Example S2)	100.0 g
Zeolite A (Wessalith P)	20.0 g
Ascorbic acid, powder	37.0 g
$NaHCO_3$	36.8 g
Release agent	3.0 g
Free-flowing agent	3.0 g

Example F2

Preparation of a Quick-Dissolving Compacted Mixture Without Effervescent Additive

Powder concentrate (according to Example S2)	100.0 g
water-soluble surfactant (Pluriol BL 500)	90.0 g
Release agent	7.0 g
Free-flowing agent	3.0 g

Example F3

Preparation of a Compacted Mixture

Powder concentrate (in accordance with Example S5)	100.0 g
Compacting aid[1)]	90.0 g
Release agent	7.0 g
Free-flowing agent	3.0 g

[1)]selected from: Avicel PH 102, Vivapur 105, FlowLac, Maltex 20, Cellactose or mixtures thereof

Example F4

Preparation of Stabilized Compacted Mixtures

Base Formula:

Powder concentrate (according to Example S5)	100.0 g
Compacting aid	50.0 g

-continued

Stabilizer	cf. Table I
Release agent	7.0 g
Free-flowing agent	3.0 g

TABLE I

Stabilizer	Component	Amount (g)
A	Zeolite A	40
	PEG 4000	40
C	Ascorbic acid[1)]	40
D	PEG 4000	20
	Ascorbic acid	20
E	Zeolite A	20
	Ascorbic acid	20
F	Zeolite A	20
	Ascorbic acid	3
	PEG 4000	17
G	Zeolite A	10
	Ascorbic acid	1.5
	PEG 4000	8.5
H	Zeolite A	7
	Ascorbic acid	1
	PEG 4000	6

[1)]In each case L-ascorbic acid

We claim:

1. A dry microorganism culture which comprises at least one microorganism species in carrier-bound form, wherein the culture is present in the form of particles which

a) have a particle size of at least about 0.1 mm and

b) comprise from about 10^{10} to 10^{12} cfu/g of at least one microorganism species;

c) have a water activity a_w of less than 0.15: and

d) are compressed.

2. A microorganism culture as claimed in claim 1, wherein the particles have been compressed under the action of a linear force from about 5 to 15 kN/cm or a pressure from about 90 to 160 MPa.

3. A microorganism culture as claimed in claim 1, wherein the compressed particles comprise compacted broken material having a diameter of from about 0.1 mm to about 2 mm.

4. A microorganism culture as claimed in claim 1, wherein the compressed particles comprise tablets having a diameter of from about 2 to 50 mm and a ratio of diameter to thickness of from about 1:0.1 to about 10:1.

5. A microorganism culture as claimed in claim 1, wherein it comprises, a further component, an effervescent additive.

6. A microorganism culture as claimed in claim 1, wherein, as carrier, it comprises at least one matrix material for embedding the microorganism cells with or without at least one further cell-stabilizing additive.

7. A microorganism culture as claimed in claim 1, wherein it comprises at least one lactic-acid-producing bacterial species.

8. A microorganism culture as claimed in claim 7, wherein the bacterial species is selected from bacteria of the genus Lactobacillus sp.

9. A process for producing a dry microorganism culture, comprising at least one microorganism species in carrier-bound form and having a water activity a_w of less than 0.15, which process comprises,

a) dissolving or suspending at least one substance suitable for forming a carrier in a liquid comprising at least one microorganism species,

b) drying the resultant mixture in a spray-dryer, for the spray-drying use being made of a conditioned dried gas

having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C., and

c) removing the dried material from the spray dryer, this dried material having an exit temperature of from about 45 to 75° C.

10. A process as claimed in claim **9**, wherein, in a further stage d), the dry material is subjected to a further drying at a temperature in the range from about 15 to 50° C. in a gas atmosphere or in vacuo and/or at least one desiccant is added.

11. A process as claimed in claim **9**, wherein, as dry material, a powder concentrate having a content of viable microorganisms of from about $5 \cdot 10^8$ to $1 \cdot 10^{12}$ cfu/g is obtained.

12. Dry compressed microorganism culture according to claim **1**, obtained from a powder concentrate of microorganism culture dried in a spray-dryer, for the spray-drying use being made of a conditioned dried gas having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C.

13. A process for preparing a dry microorganism culture as claimed in claim **1**, which comprises

i) producing a powder concentrate of the microorganism culture by carrier-bound spray-drying, carrier-bound freeze-drying or carrier bound fluidized-bed drying,

ii) with or without admixing the powder concentrate with one or more coformulants and

iii compacting or tableting this mixture.

14. A process as claimed in claim **13**, wherein the compacted powder concentrate from stage iii) is broken, with or without classification.

15. A process for preparing a dry agglomerated microorganism culture, which comprises

i) preparing a powder concentrate of the microorganism culture by carrier-bound spray-drying, carrier bound freeze drying or carrier-bound fluidized-bed drying which powder concentrate has a water activity a_w of less than 0.15,

ii) with or without admixing the powder concentrate with one or more coformulants and

iii) agglomerating this mixture.

16. A process as claimed in claim **13**, wherein the spray-drying is performed in a spray-dryer in which a conditioned dried gas is employed having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C.

17. A starter culture for foodstuffs and feedstuffs comprising a microorganism culture as claimed in claim **1**, or prepared by a process for producing a dry microorganism culture, comprising at least one microorganism species in carrier-bound form, which comprises

a) dissolving or suspending at least one substance suitable for forming a carrier in a liquid comprising at least one microorganism species,

b) drying the resultant mixture in a spray-dryer, for the spray-drying use being made of a conditioned dried gas having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C., and

c) removing the dried material from the spray dryer, this dried material having an exit temperature of from about 45 to 75° C.

18. A foodstuff or feedstuff obtainable by using a microorganism culture as claimed in claim **1** or prepared by a process for producing a dry microorganism culture, comprising at least one microorganism species in carrier-bound form, which comprises

a) dissolving or suspending at least one substance suitable for forming a carrier in a liquid comprising at least one microorganism species,

b) drying the resultant mixture in a spray-dryer, for the spray-dryer use being made of a conditioned dried gas having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C., and

c) removing the dried material from the spray-dryer, this dried material having an exit temperature of from about 45 to 75° C.

19. A process as claimed in claim **15**, wherein the spray-drying is performed in a spray-dryer employing a conditioned dried gas having a dew point of less than about +5° C., heated to a temperature in the range of above about 80° C.

20. A powder concentrate of a microorganism culture comprising from about 4×10^{11} to 10^{12} cfu/g of at least one microorganism species and having a water activity a_w of less than 0.15.

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EXHIBIT F

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(54) **Title:** NEW COMPOSITION

(57) **Abstract:** The invention describes a sports drink, which in addition to conventional additives contains viable lactobacilli having a positive effect on human intestinal mucosa. The sports drink preferably also contains micronutrients and proteins. The invention also refers to a tablet for the preparation of such a sports drink, containing viable freeze-dried lactobacilli in combination with micronutrients. In addition to providing liquid and nutrients replacement, the sports drink also relieves the stress symptoms, which are associated with long physical exercise.

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NEW COMPOSITION

The present invention refers to a sports drink which should be taken in connection with training and competition, especially in so-called endurance sports, such as skiing, marathon running and bicycling.

BACKGROUND OF THE INVENTION

It is generally known that physical exercise requires an increased and nutritionally adequate liquid intake. Dehydration rapidly decreases the capacity of an individual, but in hard training and competition the administration of salts and carbohydrates is also required in order to maintain the fluid balance, a proper salt balance and the energy level.

There is on the market today a large number of fluid and/or energy providing beverages. So called sports drinks are normally intended to be taken directly during the physical exercise to meet with the loss of fluids and salts of the body. A sports drink can be hypotonic, that is have a lower content of salts and sugars than the human body fluid, which means that it is quickly taken up by the body. Such a beverage is well fitted for short training sessions. An isotonic sports drink, that is having about the same concentration of salts and sugars as the human body, may well be used during harder and longer training sessions. A conventional sports drink contains in addition to water, carbohydrates, such as different sugars, in an amount of 4-8 %, salts and minerals.

There are also different types of nutritional additives based on vitamins, minerals and other antioxidants, or in other ways stimulating substances such as caffeine or ginseng, which can either be provided in a sports drink or in the shape of tablets or powder or any other conventional form such as an energy cake.

In connection with physical exercise it is now generally believed that there is also an increased demand of proteins and many nutritional additive products therefore also contains one or more amino acids or proteins. This is especially true for products, which are used by body-builders and other strength sports performers.

When practising an endurance sport or exercising physically during a long period of time the body will be in a state of

stress. This implies an increased flow of blood to the muscles, increased production of free radicals, and increased level of the so called stress hormones adrenaline, noradrenaline and cortisol. This state of stress also leads to gastrointestinal problems for many people practising said sports, such as marathon runners and hard training athletes. The gastro-intestinal problems can be manifested in many different ways, such as constipation, diarrhea, stomach ache, cramp or nausea (Nancy Rehrer et al., Gastrointestinal complaints in relation to dietary intake in triathletes, International Journal of Sport Nutrition, 1992, 2, 48-59). Competitive long-distance running is also said to induce gastrointestinal blood loss which may contribute to iron deficiency, runner´s anaemia (James G. Stewart et al., Gastrointestinal Blood Loss and Anaemia in Runners, Annals of Internal Medicine, 1984, Vol. 100, No. 6, 843-845). Said intestinal bleedings might be due to a weakened intestinal mucosa.

PRIOR ART

GB 2 335 134 A, Stalplex Ltd, discloses a carbonated sport beverage comprising fruit juice, carbohydrates and a soluble whey protein hydrolysate. The beverage is to be used by people engaged in physical activities. Nothing is, however, stated about the optional effects of the beverage.

WO 89/08405, Nils Molin et al., describes a nutrient composition for administration to patients in feeding by tube or for use as a health drink. The nutrient composition comprises fermented oat-flour in combination with lactobacilli, optionally also soya flour or skim milk powder and supplementary mineral substances and vitamins. A nutrient composition should cover the total nutrient requirements and should contain carbohydrates, proteins and fat. The amount of antioxidants will on the other hand be fairly low.

There is therefore still a need for an improved sports drink which can allieviate the symptoms of prolonged physical activity.

DESCRIPTION OF THE INVENTION

The purpose of the invention is to provide a sports drink

which in addition to a nutrient and fluid supplementation before or after physical activity in order to build up and recover, respectively, the energy and fluid levels of the body, also relieves the symptoms of stress. It has now surprisingly been shown that viable lactobacilli can be mixed with micro-nutrients, carbohydrates, salts and proteins, without negative effects on e.g. antioxidants, to a beverage having a good taste and a good shelf-life.

The invention refers to a sports drink which is characterised in containing viable lactobacilli having a positive effect on human intestinal mucosa.

The invention especially refers to a sports drink which comprises micronutrients in combination with conventional additives for a sports drinks, which is characterised in that it also contains viable lactobacilli having a positive effect on human intestinal mucosa.

Lactobacilli which are suitable to use in accordance with the invention comprise those different strains of different species which have a positive effect on human intestinal mucosa. Such an effect involves an ability to colonise in the intestines and thereby protect the intestinal mucosa, for instance by initiating the production of mucin or short chain fatty acids. Examples of strains having this ability can be found among the following species *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus*.

A suitable species of *Lactobacillus*, which can be used according to the invention, is a *Lactobacillus plantarum* being able to adhere to the intestinal epithel and colonise in the intestines. Particularly suitable strains of this species comprise a mannose specific adhesin, such as described in WO 96/29083, and are part of a cluster of *L. plantarum* having more than 70 % similarity to *L. plantarum* 299, deposited on July 2, 1991 at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany under the accession number DSM 6595, with respect to REA, that is restriction enzyme analysis of the total chromosomal DNA. A preferred strain is *L. plantarum* 299v, deposited on March 16, 1995 under the accession number DSM 9843.

Other strains of interest which can be used in a sports drink according to the invention are the probiotic strains *Lactobacillus rhamnosus* 271 (DSM 6594), *Lactobacillus rhamnosus* GG (ATCC 53103), *Lactobacillus casei rhamnosus* LB 21, *Lactobacillus casei* Shirota, *Lactobacillus johnsonii* Lj1, *and Lactococcus lactis* L1A.

Micronutrients in this context refers to vitamins, minerals and other additives having an antioxidising or stimulating effect. As examples can be mentioned:

Ascorbic acid or vitamin C, which acts as an antioxidant but also takes part in hydroxylation reactions. Lack of ascorbic acid can result in such symptoms as tiredness, weakness and also loosening of the teeth. According to the Swedish nutritional recommendations the intake should be 35-60 mg per day;

Carotenoids acts as antioxidants. The carotenoid group includes both carotenes and xantophylles. The difference between them is the occurrence of oxygen in the xanthophyll molecule. Examples of carotenoids are α-carotene, β-carotene, γ-carotene, lycopene, lutein, chryptoxanthin, astaxanthin, canthaxanthin, and zeaxanthin. About 50 of approximately 500 naturally occurring carotenoids are precursors of retinol. E.g. β-carotene is a provitamin to vitamin A. The vitamin is needed for the sight, growth, reproduction and the normal differentiating and stability of the epithel tissues. A-vitamin is also considered to be of importance for the immune defence. The recommended daily intake of retinol equivalents for men is 1000 μg and for women 800 μg per day. Astaxanthin is a powerful antioxidant, which has proven to give an increased antibody production;

Vitamin E, natural or synthetic, is considered to function as an antioxidant and by that contribute to the stability of the cell membranes by protecting the polyunsaturated fatty acids in the lipids of the membranes. The recommended daily intake is 10 mg α-tocopherol equivalents for men and 8 mg for women;

Vitamin B6 is the common name for pyridoxine, pyridoxal and pyridoxamine. Lack of vitamin B6 is uncommon and in that case occurs together with lack of other B-vitamins. Symptoms are for instance cramps and anemia. The recommended intake is 1.5-2 mg per day and the need is proportional to the amount of protein, which is metabolizsed in the body;

Thiamin, vitamin B1, acts as a coenzyme in several enzymes of importance for the energy metabolism in e. g. the citric acid cycle. A lack thereof can cause beriberi, which involves disorders of the nerve system, heart and digestion organs. An intake of 0.5 mg per 1000 kcal is believed to give tissue saturation, the recommendations are 1 mg and above;

Riboflavin, vitamin B2, takes part in the conversion of tryptophane into niacin. A lack thereof is generally connected to a deficiency of other B-vitamins and symptoms of this deficiency can be hypersensitivity to light and reddening mouth mucosa. Recommended intake is 1.6 mg per day for men and 1.3 mg for women;

Niacin, nicotinic acid, is a part of coenzymes and constitutes a part of NAD and NADP, which are necessary in the conversion of glucose, amino acids and fat. Lack thereof can result in pellagra. Characteristics are changes in the skin, gastro-intestinal system. Recommended intake of niacin should be proportional to the consumption of energy and is about 13-18 mg per day;

Cobalamin, vitamin B12, acts as a coenzyme in transferring one-carbon groups and takes part, together with folic acid, in the formation of active methyl groups. Vitamin B12 deficiency can result in pernicious anaemia and also cause nerve damages. According to Swedish nutritional recommendations 3 μg per day is regarded to cover the requirement;

Folacin, folic acid, acts as a coenzyme and transfers one-carbon fragments in amino acid synthesis and nucleic acid synthesis. Folacin deficiency results in impaired cell division, disordered protein conversion and megaloblastic anaemia. Recommended intake of folacin is 200 μg per day;

CoQ10 or coenzyme Q10 is an antioxidant protecting against free radicals, in particular oxidation of lipids is prevented. In general about 100 mg per day is given;

Flavonoides, which are present in vegetable food, are powerful polyphenolic antioxidants preventing oxidation of lipoproteins and reducing the risk of coronary heart diseases;

Copper is part of enzymes, which are necessary for the energy metalbolism of the cells, synthesis of connective tissue, synthesis of neuropeptides and in the body defence against free

radicals. Deficiency symptoms in adults are dysarrhytmia and other changes of the heart function. Recommended intake is 1.2 mg for adults;

Magnesium is the prosthetic group of many enzymes and also functions as an activator. ATP:ase is for instance magnesium dependent and is part of inter alia the contraction of the muscle cells, the Na/K-pump. Magnesium is also needed for nucleic acid and protein synthesis. In addition, the function of certain nerve cells depends on magnesium. Recommended intake of magnesium is 280 mg per day for women and 350 mg for men;

Manganese inter alia takes part of the synthesis of proteins, mucopolysaccharides and cholesterol. Possible symptoms of manganese deficiency in man are changes of the skin and hypocholesterolemia. Recommended intake is 25 mg per day;

Selenium mainly is part of the enzyme glutathioneperoxidase and protects the cells from oxidative lesions. The recommended intake of selenium is 40 μg for women and 50 μg for men. The intake should not exceed 5 μg per kg body weight and day. Selenium deficiency can cause a heartmuscle disease;

Zinc is a part of many enzymes. Zinc deficiency can be manifested as growth retardation and changes of the skin. Recommended intake of zinc according to the Nordic nutritional recommendations is 7 mg for women and 9 mg for men. Too high an intake negatively effects the metabolism of other trace elements. The zinc intake should not exceed 45 mg for adults and 25 mg for children;

Chromium is the biological active component of the glucose tolerance factor, which potentiates the insulin activity. A supplement of chromium could improve the efficiency of insulin.

The invention especially refers to a sports drink containing micronutrients selected from the group consisting of ascorbic acid, vitamin E, carotenoids, pyridoxine, thiamine, riboflavine, niacin, cobalamine, folacin, Q10, flavonoides, copper, magnesium, manganese, selenium, zinc, and chromium. A synergistic effect can be expected for a mixture of the stated compounds. It is for instance well known that vitamin C, vitamin E and selenium have a synergistic effect.

Salts of sodium and potassium are necessary to administer after exercise in order to recover the salt balance; this is

true especially in a warm climate with attendant perspiration. Acute salt deficiency causes nausea and impaired nerve impulses, which inter alia is manifested in a swaying and stumbling gait. Moderately high levels of sodium, such as up to 50-60 mmol/l, and also some potassium should therefore be part of the composition according to the invention in order to compensate for losses through perspiration.

A preferred combination of micronutrients and salts in a sports drink according to the invention is, per 1000 g sports drink:

ascorbic acid	500-1200 mg
vitamin E	250-375 mg
β-carotene	15-25 mg
pyridoxine	15-25 mg
sodium	20-60 mg
potassium	60-100 mg
copper	0.5-1.5 mg
magnesium	120-175 mg
manganese	1-3 mg
selenium	0.05-0.15 mg
zinc	5-15 mg

According to a preferred aspect the invention refers to a sports drink, which in addition to micronutrients and live lactobacilli also comprises proteins.

Proteins, which are suitable to use in a sports drink according to the invention, should be water soluble, acid stable and heat stable. As examples can be mentioned different milk proteins, especially whey proteins or whey protein hydrolysates. Whey protein isolates are one of the protein sources supplying most essential amino acids and branched amino acids per g of nitrogen. A preferred whey protein is highly soluble in water and forms low viscous, homogenous and comparatively clear solutions after mixing with water. Another possible source of proteins is bovine colostrum. Different amino acids, especially branched amino acids which are taken up by the muscles, can also be added to give a corresponding supplement of energy. Essential, branched amino acids, which can be added with the whey protein, are leucine, isoleucine and valine.

The sports drink of the invention also contains carbohydrates and salts in an aqueous solution, preferably flavoured with a fruit juice concentrate and aromas.

Preferred carbohydrates are the so-called slow carbohydrates, having a low glycemic index. GI, glycemic index, is a measure of how quickly the carbohydrates of a food enter the blood. Fructose is the monosaccharide having the lowest glycemic index and is particularly preferred. If you wish to prepare a beverage which is hypotonic it could, however, be adequate to use a so called polysugar, maltodextrin, which has a high carbohydrate concentration but a low osmotic pressure, in an amount of 2-20 dextrose equivalents. For different reasons it might, however, be preferred to use the slow carbohydrate in admixture with a carbohydrate having a high glycemic index, for instance other mono- or disaccharides, such as glucose and saccharose.

If the monosaccharide content is lower than 50 g per 1000 g, an isotonic beverage is obtained; at higher contents a hypotonic beverage. If this beverage also contains salt the amount will be different.

Aromas can for instance be produced from different concentrated fruit juices and is preferably used when the sport drinks contain proteins, especially whey proteins, which might otherwise give the beverage a special, bitter taste.

A sports drink according to the invention can for instance per 1000 g contain:

whey protein	15-60 g
carbohydrates	40-150 g
micronutrients	1-2 g
probiotic strain of *Lactobacillus*	$5 \cdot 10^7$ - $5 \cdot 10^8$ cfu/ml

According to a preferred aspect of the invention the sports drink contains per 1000 g:

whey protein isolate	15-60 g
mono- and disaccharides	40-150 g
micronutrients	1-2 g
L. plantarum DSM 9843	$5 \cdot 10^7$ - $5 \cdot 10^8$ cfu/ml

According to another aspect the invention refers to a nutritional additive comprising micronutrients in combination with freeze-dried, viable lactobacilli, especially in the shape of a tablet or a powder. This nutritional additive can also be an energy cake or some other conventional nutritional product wherein the lactobacilli can be preserved alive.

The invention especially refers to a tablet for preparing of a sports drink as above, which tablet comprises micronutrients in combination with freeze-dried, viable lactobacilli. Such a tablet can be prepared by mixing a freeze-dried culture, in an amount of 10-20 % by weight of the total composition, of selected lactobacilli with micronutrients, 1-3 %, and an adequate tablet making material which allows for the use of a comparatively low punching pressure at the tabletting, for instance according to the process which is described in WO 97/07822. By using a reduced punching pressure it will be possible to maintain 90-95 % of the viability of the bacteria. Possible tablet making materials are poly- and oligosaccharides, especially based on fructose, calcium diphosphate, micro-crystalline cellulose and maltodextrine as a filler, xanthan as a slime forming agent and magnesium stearate as a lubricant. The tablet can also be an effervescent.

By mixing a tablet prepared in this way with water or with water and carbohydrates, with or without proteins and salts, or with a conventional sports drink, a sports drink according to the invention for direct consumption is obtained. It is of course also possible to make the mixture in vivo, that is to eat the tablet in connection with the intake of the fluid.

A hypertonic sports drink according to the invention containing proteins, carbohydrates and lactobacilli can preferably be taken before or after competition or training in an amount of ½ to 1 litre per day. This corresponds to a total daily consumption of $3 \cdot 10^{10}$ - $5 \cdot 10^{10}$ cfu/ml. The beverage is, owing to its content of proteins and lactobacilli, less apted for being taken during the physical exercise. There is, however, nothing that prevents the use of a beverage consisting of hypertonic amounts of carbohydrates and salts and micronutrients in combination with lactobacilli and optimal flavouring additives also during the physical exercise as such.

The invention in addition refers to the use of lactobacilli for the preparation of a sports drink to be used to prevent and treat stress symptoms, disturbances of the gastrointestinal tract, and lesions of the mucose membrane of the intestines. It has been shown that a regular intake of a sports drink according to the invention has a positive effect on the stress related gastrointestinal problems. By taking this beverage the negative effect of stress is reduced, the risks of disorders in stomach and intestines are decreased and, especially, the risk of disorders of the intestinal mucosa is reduced.

It is probable that the most favorable effect on the intestinal mucosa is obtained with a sports drink containing a combination of whey protein, micronutrients and lactobacilli.

EXAMPLES

Example 1. Hypertonic sports drink

For the preparation of a hypertonic sports drinks the following constituents are used

whey proteins	22.5 g
fructose	20 g
glucose	60 g
saccharose	40 g
oatbase	50 g
micronutrients	1.5 g
fruit juice concentrate	15 g
aromas	1.5 g
water	q. s. ad 1000 g

citric acid for adjusting pH to 3.4

All the constituents are weighed. The whey protein, Lacprodan® DI-9213 (MD-Foods, Viby, Denmark), is mixed with water and then homogenised on ultraturrax, position 2 (13,000 rpm), for about 1 minute. Then fructose, glucose and saccharose, and aromas are added, mixed and heated during stirring to 90°C for 5 minutes in a water-bath. The fruit juice concentrate could be from lemon-lime, black current or tropical, all from Skånemejerier Ekonomisk förening, Malmö. The aroma has a lemon-lime taste and has been obtained from Quest International, Lund. The batch is then cooled to room temperature and the oatbase which contains *Lactobacillus plantarum* 299v in an amount of

$1\text{-}2\cdot10^9$ cfu per ml, and micronutrients are added. After mixing a well tasting beverage is obtained which is aseptically packed and then cool stored.

The micronutrients and the proportion between them are, calculated on the metal when applicable, as follows

ascorbic acid	800 mg
vitamin E	320 mg (400 IE)
β-carotene	20 mg
pyridoxine	20 mg
copper ($CuSO_4$)	1 mg
magnesium (MgO)	150 mg
manganese (Mn_2SO_4)	2 mg
selenium (Na_2SeO_3)	100 μg
zinc ($ZnSO_4$)	10 mg

and constitutes a powder mixture.

The oatbase used above is a storage solution of lactobacilli and is prepared from oat meal, malt flour and water which are mixed and heated at different temperature ranges to give a proper substrate for the selected strain of *Lactobacillus*. In this case *Lactobacillus plantarum* DSM 9843 is used, which is added to the cooled oats mixture and which after fermentation gives a content of about $2 \cdot 10^9$ cfu/ml oatbase.

The sports drink thus prepared contains per 100 g

protein	2.1 g
fat	0.1 g
carbohydrates	13 g
sodium	2.3 mg
potassium	6.4 mg
lactobacilli	$5\cdot10^7$ cfu/ml

The shelf-life of the prepared sports drink has turned out to be at least 4 weeks when stored in a refrigerator, +4 to +8°C.

Example 2. Hypertonic sports drink

For the preparation of another hypertonic sports drinks the following constituents are used

Lacprodan® DI-9213	45 g
fructose	120 g

oatbase	50 g
micronutrients	1.5 g
fruit juice concentrate	20 g
aromas	1.5 g
water	q. s. ad 1000 g

The same procedure as described in Example 1 was followed, the same micronutrients and lactobacilli were added and the sports drink thus prepared contained per 100 g

protein	4 g
fat	0.1 g
carbohydrates	13 g
sodium	4.5 mg
potassium	8.6 mg
lactobacilli	$5 \cdot 10^7$ cfu/ml

Example 3. Tablet

The following ingredients are mixed in the stated weight proportion

L. plantarum DSM 9843, freeze-dried	20 %
inulin	78 %
micronutrients	2 %

and compressed into tablets. The micronutrients have the composition stated in Example 1 above. 1 tablet could preferably be taken together with about ½ litre of fluid.

Example 4. Tablet

In the same way as in Example 3 the following ingredients are mixed, giving a tablet having improved solubility properties.

L. plantarum DSM 9843, freeze-dried	10 %
inulin	85 %
micronutrients	2 %
magnesium stearate	1 %
xanthan	2 %

BIOLOGICAL TESTS

Effects of *L. plantarum* DSM 9843 in a rat intestinal bleeding model

36 male Sprague-Dawley rats were used for the experiment. They were housed in separate metabolic cages to collect stool individually for evaluation. The animals were kept at room temperature, 22°C, with a controlled 12 h light/dark cycle and had free access to standard rat chow and drinking water. There were three different experimental groups with 12 rats in each. Group 1 was the negative control which received no treatment. Group 2 was the positive control which was given DSS, dextrane sulphate, in order to induce intestinal bleeding. The rats in Group 3 were treated with DSS + *L. plantarum* DSM 9843. All rats underwent sham operation for insertion of a pump for a later experiment under anaesthesia. On day 1 the rats were operated and on the following 6 days DSS was administered. Group 1 had free access to tap water and Groups 2 and 3 received ad libitum 5 % (w/v) DSS (ICN Biomedicals Inc. Aurora,Ohio, USA) dissolved in the drinking water. The Group 3 rats were fed enterally with an oatmeal drink containing $1x10^{10}$ cfu/ml of *L. plantarum* DSM 9843 twice daily in a volume of 2 ml through an oro-gastric tube.

The following results were obtained

Table 1. Mean of Disease Activity Index

day	Group 1	Group 2	Group 3
1	0.3	0.4	0.2
2	0.1	0.5	0.6
3	0.0	0.5	0.5
4	0.0	0.8	0.9
5	0.1	1.5	1.0*
6	0.2	2.3	1.8*
7	0.0	2.8	2.3*

* denotes $P<0.05$ compared to Group 2, that is the DSS group

The Disease Activity Index, which is a combination of scores for weight loss, stool consistency and bleeding, divided by 3, was

scored as below

Table 2. Scoring of Disease Activity Index

Score	Weight loss, %	Stool consistency	Bleeding
0	none	normal = well-formed pellets	negative
1	1-5		
2	5-10	loose stool = pasty, not sticking to the anus	hemoccult +
3	10-20		
4	>20	diarrhea = liquid stool sticking to the anus	gross bleeding

The results of this test show that administration of *Lactobacillus plantarum* to the intestines has a positive effect on the intestinal mucosa.

Pilot study with the Sports drink of Example 1

7 hard training persons, No. 1-7, were given the sports drink according to Example 1 and were told to take ½ to 1 liter per day before or after the physical exercise for a period of 4 weeks. They were also asked to give their views on optional differences as to gastrointestinal behavour, recovery, sense of well-being, ache after training and optional other aspects.

Person No.1 is a long-distance runner training more than 7 times a week. He experienced no gastrointestinal problems during the test. From day 5 he finds that his capacity and total well-being had increased. After 28 d the ache after training has decreased.

Person No. 2 is a handball player, and mentions less ache after training and increased well-being.

Person No. 3 is an athlete. In addition to an increased well-being she believes that the recovery is quicker and sometimes the performance better.

Person No. 4 is a marathon runner. He liked the drink and found it of value during hard training, but finds the test

period of 4 weeks to be too short. He had rarly any gastrointestinal problems - only during extremely intensive trining sessions.

Person No. 5 is a marathon runner who mentions less ache after training, increased well-being and good recovery. Above all she is happy with an improved gastrointestinal behaviour, previous diarrhea after hard training has disappeared and she has no longer any gastrointestinal problems.

Person No. 6 is an athlete, running 400 and 800 m. She found the drink to increase her well-being, reduce the ache after training, reduce the disease frequence, increase the recovery rate.

Person No. 7 is an athlete, running middle distances. She experienced an improved gastrointestinal behaviour, an increased recovery rate, a reduced infection frequency and in general an improved total well-being.

The conclusions from this pilot study are that those individuals who had gastrointestinal problems in connection with hard training experienced an clear improvment; the reduction of the ache after training was also a common feature.

Studies in progress

In order to closer investigate the effects of the sports drink of the invention different tests are to be performed. In one study different parameters, such as antioxidative capacity in blood, gastrointestinal function and optional effects on the microflora, are to be studied on healthy persons having a high tempo in their working life. Another study is to be made with hard training athletes, in which maximum oxygen uptake, lactate treshold values, anaerobic capacity and power of endurance are to be tested.

In connection with a marathon competition the runners will be asked to participate in a study to evaluate the effects of the sports drink of the invention in reducing the occurrence of faecal blood.

Applicant's or agent's file reference	International application
HeL/UB 43541	PCT/SE00/01024

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 3, line 38 - 39.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1B
D-38124 Braunschweig
Germany

Date of deposit	Accession Number
16 March 1995	DSM 9843

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application 2 2 -05- 2000	☐ This sheet was received by the International Bureau on:
Authorized officer Inger Willén	Authorized officer

Form PCT/RO/134 (July 1998)

CLAIMS

1. A sports drink, characterized in containing viable lactobacilli having a positive effect on human intestinal mucosa.

2. A sports drink comprising micronutrients in combination with conventional additives for sport drinks, characterized in containing in addition viable lactobacilli having a positive effect on human intestinal mucosa.

3. A sports drink according to claim 1 or 2, characterised in containing one or several strains of *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus* in a therapeutically effective amount.

4. A sports drink according to claim 2 or 3, wherein the micronutrients are selected from the group consisting of ascorbic acid, vitamin E, carotenoids, pyridoxine, thiamine, riboflavin, niacin, cobalamin, folacin, Q10, flavonoids, copper, magnesium, manganese, selenium, zinc and chromium.

5. A sports drink according to any of claims 2-4, characterised in containing per 1000 g

ascorbic acid	500-1200 mg
vitamin E	250-375 mg
β-carotene	15-25 mg
pyridoxine	15-25 mg
sodium	20-60 mg
potassium	60-100 mg
copper	0.5-1.5 mg
magnesium	120-175 mg
manganese	1-3 mg
selenium	0.05-0.15 mg
zinc	5-15 mg

6. A sports drink according to any of claims 1-5, which

comprises proteins, optionally in combination with amino acids.

7. A sports drink according to claim 6, wherein the protein is a whey protein or whey protein hydrolysate.

8. A sports drink according to any of claims 1-7, which comprises carbohydrates having a low glycemic index, optionally in combination with carbohydrates of a high glycemic index.

9. A sports drink according to any of claims 2-8, characterised in containing per 1000 g

whey proteins	15-60 g
carbohydrates	40-150 g
micronutrients	1-2 g
probiotic strain of *Lactobacillus*	$5 \cdot 10^7$ - $5 \cdot 10^8$ cfu/ml

10. A sports drink according to any of claims 2-9, characterised in containing per 1000 g:

whey protein isolate	15-60 g
mono- and disaccharides	40-150 g
micronutrients	1-2 g
L. plantarum DSM 9843	$5 \cdot 10^7$ - $5 \cdot 10^8$ cfu/ml

10. Tablet for the preparation of a sports drink according to any of claims 2-9 in vivo or in vitro, characterised in that it comprises micronutrients in combination with freeze-dried, viable lactobacilli.

11. Use of lactobacilli for the preparation of a sports drink according to any of claims 1-10 to prevent and treat stress symptoms, gastrointestinal disturbances, and lesions of the mucose membrane of the intestines.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01024

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A23L 2/52
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E,X	EP 1020123 A1 (SITIA-YOMO S.P.A), 19 July 2000 (19.07.00)	1-11
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Y	AU 199737828 B2 (CALPIS CO., LTD. ET AL), 25 February 1998 (25.02.98)	1-11
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Y	GB 2335134 A (STALPLEX LIMITED), 15 Sept 1999 (15.09.99)	1-11
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Y	WO 9846091 A1 (OY ITARA HK AB), 22 October 1998 (22.10.98)	1-11
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	10 -10- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Solveig Gustavsson/EÖ Telephone No. +46 8 782 25 00

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9406412 A1 (THE PROCTER & GAMBLE COMPANY), 31 March 1994 (31.03.94)	1-11
Y	GB 1157135 A (KABUSHIKI KAISHA YAKULT HONSHA), 2 July 1969 (02.07.69)	1-11
Y	WO 8908405 A1 (MOLIN, NILS), 21 Sept 1989 (21.09.89)	1-11
Y	EP 0856259 A1 (SITIA-YOMO S.P.A.), 5 August 1998 (05.08.98)	1-11
Y	EP 0916270 A2 (ENORGYBALANCE AG IN GR.), 19 May 1999 (19.05.99), claim 1	1-11
A	File WPI, Derwent accession no. 1997-014808, Kyosho Juan KK et al: "Prepn. of fermented soln. of lactic used as food additive - comprises adding water to rice bran and/or unpolished rice, inoculating lactic acid bacterium, contacting with gas and applying ultrasonic waves"; & JP,A,8280341 19961029 DW199702	1-11

EXHIBIT G

United States Patent [19]

Brassart et al.

[11] **Patent Number: 5,603,930**

[45] **Date of Patent: Feb. 18, 1997**

[54] *LACTOBACILLUS JOHNSONII* CNCM I-1225

[75] Inventors: **Dominique Brassart**, Bussigny; **Anne Donnet**, Saint-Legier; **Harriet Link**, Vevey; **Olivier Mignot**, Blonay; **Jean-Richard Neeser**, Savigny; **Florence Rochat**, Montreux; **Eduardo Schiffrin**, Crissier, all of Switzerland; **Alain Servin**, Chatenay-Malabry, France

[73] Assignee: **Nestec S.A.**, Vevey, Switzerland

[21] Appl. No.: **455,562**

[22] Filed: **May 31, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 430,264, Apr. 28, 1995, Pat. No. 5,494,664, which is a continuation of Ser. No. 84,525, Jun. 29, 1993, abandoned.

[30] **Foreign Application Priority Data**

Jul. 6, 1992 [EP] European Pat. Off. 92810516

[51] **Int. Cl.**[6] **C12N 1/20**; A01N 63/08

[52] **U.S. Cl.** **424/93.45**; 435/252.9; 435/854; 426/61; 426/583; 426/584; 426/588; 426/580

[58] **Field of Search** 435/252.9, 854; 424/93.45; 426/61, 583, 587, 588, 580

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(List continued on next page.)

Primary Examiner—Irene Marx

Attorney, Agent, or Firm—Vogt & O'Donnell, LLP

[57] **ABSTRACT**

Lactobacillus johnsonii strain CNCM I-1225 adheres to Caco-2 cells and inhibits adhesion thereto by enterovirulent and enteroinvasive pathogens.

10 Claims, No Drawings

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LACTOBACILLUS JOHNSONII CNCM I-1225

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation application of Ser. No. 08/430,264, filed Apr. 28, 1995, now U.S. Pat. No. 5,494,664, which is a continuation application of Ser. No. 08/084,525, filed Jun. 29, 1993, now abandoned.

This invention relates to a biologically pure culture of a lactic acid bacterium strain, to a composition containing this strain and to the use of the strain.

European Patent Application Publication No. 199 535 (Gorbach and Goldin) proposes a bacterial strain identified in the first instance as being a *Lactobacillus* (*L*.) *acidophilus*, but then as bearing more of a resemblance to *L. casei* subs. *ramnosus* (cf. M. Silva et al. in Antimicrobial Agents and Chemotherapy, 31, No. 8, 1231–1233, 1987), which shows good adhesion to the cells of the mucus of the small intestine and which lends itself to therapeutic applications. This strain, baptized "strain GG" and lodged in the ATCC (American Type Culture Collection) under No. 53103, may be used in conjunction with a pharmaceutically acceptable support, more particularly in food products, above all in acidified milk products of the yogurt type for example.

Other strains of the same type have long been used in analogous products and with analogous objectives. However, there is a need for particularly high-performance strains of this type which could be clearly identified, which would have indisputable advantages and which would enrich the range of available strains.

The problem addressed by the present invention was to satisfy this need.

SUMMARY OF THE INVENTION

The present invention provides a biologically pure culture of a strain of lactic acid bacterium selected for its affinity for implantation in an intestinal flora, for its ability to adhere to intestinal cells, for its capacity for competitive exclusion of pathogenic bacteria from intestinal cells and for its capacity for immunomodulation and/or reduction of fecal enzymatic activity.

The strain in question is particularly intended for administration to human beings or animals for therapeutic or prophylactic treatment of the gastrointestinal system, more particularly as an antidiarrhoeic.

The strain may be administered in the form of a biologically pure culture, for example as such, after freezing and/or freeze-drying. The culture in question may comprise, for example, 10^8 to 10^{10} viable germs (cfu from the technical English expression "colony forming units") per g for the liquid or frozen form and 10^9 to 10^{11} cfu/g for the freeze-dried form.

The strain may also be administered in the form of a composition containing the culture and an ingestible support, more particularly a pharmaceutically acceptable support or a food product such as, for example, an acidified milk, more particularly a yogurt or a milk-based powder formulation.

DETAILED DESCRIPTION OF THE INVENTION

In a first preferred embodiment, the invention provides a culture of a strain of lactic acid bacterium selected for its affinity for implantation in the digestive tube of mice or rats with human intestinal flora.

In a second preferred embodiment, the invention provides a culture of a strain of lactic acid bacterium selected for its capacity for competitive exclusion of the pathogenic bacteria responsible for diarrhoea from intestinal cells.

Among various strains of bacteria thus selected in particular from acidified milks, particularly commercial yogurts, or from commercial cultures intended for the preparation of such milks or from the feces of infants for example, four were lodged by way of example under the Budapest Treaty on the Jun. 30, 1992 in the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 28 rue de Dr. Roux, 75724 Paris Cedex 15, France, where they were each given the respective CNCM number shown in brackets below, namely a strain of *Lactobacillus acidophilus* (CNCM I-1225), a strain of *Bifidobacterium breve* (CNCM I-1226), a strain of *Bifidobacterium infantis* (CNCM I-1227) and a strain of *Bifidobacterium longum* (CNCM I-1228).

Subsequent to deposit of strain CNCM I-1225, the taxonomic classification of *Lactobacillus acidophilus* was reorganized to include six subgroups or "genomospecies." See Fujisawa, et al., Taxonomic Study of the *Lactobacillus acidophilus* Group with Recognition of *Lactobacillus gallinarum* sp nov and *Lactobacillus johnsonii* sp nov and Synonymy of *Lactobacillus acidophilus* Group A3 with the Type Strain of *Lactobacillus amylovorous*, Int. J. Syst. bacteriol. 42:487;491 (1992). Subsequent to that taxonomic reclassification, it was determined by species-specific DNA probe ("Lbj") that strain CNCM I-1225 is a member of the newly established *Lactobacillus johnsonii* species.

Details of the morphology and general properties of these strains are given in the following:

L. johnsonii CNCM I-1225

Morphology:

Gram-positive microorganism, non-motile, non-sporing.

Isolated, fairly short and thick rodlets.

Metabolism

Microaerophilic microorganism with homofermentative metabolism giving rise to the production of L(+) and D(−) lactic acid.

Other characteristics: catalase (−) , production of CO_2 (−), hydrolysis of arginine (−).

Fermentation of sugars:

Amygdaline (+), arabinose (−), cellobiose (+), esculine (+), fructose (+), galactose (+), glucose (+), lactose (+), maltose (+/−), mannitol (−), mannose (+), melibiose (−), raffinose (+), ribose (−), salicine (+), sucrose (+), trehalose (+).

B.Breve CNCM I-1226. *B. infantis* CNCM I-1227 and *B. longum* I-1228

Morphology:

Gram-positive microorganisms, non-motile, non-sporing.

Short rodlets with rounded ends and "V" or "Y" branches.

Metabolism:

Anaerobic microorganisms with heterofermentative metabolism giving rise mainly to the formation of lactic and acetic acid.

Other characteristics: catalase (−), production of CO_2 (−), hydrolysis of arginine (−).

Fermentation of sugars

Since the sugar fermentation profile of these species is very unstable and non-reproducible, only a few sugars are always fermented, particularly D-ribose, lactose and raffinose.

Details of the particular properties for which the present strain may be selected are given below:

Implantation in an intestinal flora

Gnotoxenic mice

Two groups of axenic mice (mice without intestinal flora) are each associated with the human flora of a different donor (gnotoxenic mice). After several days' acclimatization, the intestinal flora of the mice are entirely comparable with those of the human donors from the functional, qualitative and quantitative viewpoints.

According to the invention, numerous strains have been tested for their ability to colonize the digestive tube of these mice with human flora, i.e. for their affinity for implantation in this intestinal flora.

It was found that most of the strains are not capable of colonizing these animals even after several successive inoculations, although the *L. johnsonii* strain CNCM I-1225, for example, was capable of proliferation and implantation in the digestive tube, i.e. in the intestinal flora of the mice of the two groups, even after a single inoculation.

This colonization or implantation enables the strain to be present in the feces in quantities of more than 10^6 cfu/g. This content of viable germs of the strain in the feces may be considered as necessary and/or sufficient for the metabolism of the strain to be able to modify that of the host.

It was also found that this implantation persists as long as the environment of the animals is not disturbed.

Gnotoxenic rats

Axenic rats are associated (gnotoxenic rats) with an isolated strain (*Bacteroides thetaiotamicron* FI 1, particular collection of the Centre de Recherche Nestec SA, CH-1000, Lausanne, Switzerland) of a human intestinal flora of a healthy donor intended, as will be seen hereinafter, to simulate the production of enzymes of a complete fecal flora. This association results in abundant colonization of the intestine of these rats so that the bacterium is present in the feces in quantities of approximately 10^8 cfu/g.

An implantation test of the *L. johnsonii* strain CNCM I-1225, for example, in this flora results in good co-colonization so that the strain is also present in the feces in quantities of approximately 10^8 cfu/g.

Human volunteers

The number of viable germs of *L. bulgaricus* appearing in the feces of healthy human volunteers who ate traditional yogurt prepared by fermentation of a cow's milk with a commercial culture of *L. bulgaricus* and *S. thermophilus* was determined by way of comparison.

The volunteers did not eat any fermented milk product for three consecutive periods of three weeks each except for the yogurt which they ate during the second three-week period.

In the three weeks when they ate yogurt, they did so in such a way as to ingest approximately 10^{10} *L. bulgaricus* per day which corresponded to approximately three 120 g yogurts per day. During the period of consumption of the yogurts, the feces of the volunteers were found to contain approximately 10^5 cfu of *L. bulgaricus* per g.

According to the invention, a test was conducted in the same scenario as above, but with yogurt prepared by fermentation of a milk with a commercial culture of *S. thermophilus* and *B. bifidus* supplemented, for example, with the *L. johnsonii* strain CNCM I-1225 in a concentration of the same order.

The total number of viable germs of lactobacilli in the feces of the volunteers was determined before, during and after the period of consumption of the yogurt. Values of 10^5 to 10^6 cfu/g were found before, values of more than 10^7 cfu/g during and values of 10^6 cfu/g after the period of consumption.

Accordingly, there was an increase in the total number of lactobacilli found in the feces during the period of consumption of the yogurt. The CNCM I-1225 strain was found in a significant quantity and in viable form in the volunteers. By contrast, it was eliminated in a few days after the volunteers stopped eating the yogurt.

Reduction of fecal enzymatic activity

Gnotoxenic rats

The fecal azoreductase and nitroreductase activity was determined in tests conducted with the gnotobiotic rats mentioned above. This was because the enzymes azoreductase and nitroreductase were involved in the production of carginogenic substances. A high concentration of these enzymes is associated with an increased risk of cancer of the colon.

It was found that the fecal enzymatic activity of the gnotobiotic rats with Bacteroides rose to 2.5 μg/h/mg protein for azoreductase and to 4.2 μg/h/mg protein for nitroreductase whereas, for the gnotobiotic rats with Bacteroides in the flora of which the CNCM I-1225 strain, for example, had been implanted, this enzymatic activity rose to 1.8 μg/h/mg protein for azoreductase and to 3.5 μg/h/mg protein for nitroreductase.

In addition, it was found that gnotobiotic rats with intestinal flora formed exclusively from the CNCM I-1225 strain showed no fecal azoreductase or nitroreductase activity.

In other words, the presence of the CNCM I-1225 strain in the flora of gnotobiotic rats induces a reduction in the production of certain undesirable enzymes in these animals, i.e, beneficial modifications in the metabolism of the host.

Human volunteers

Fecal nitroreductase activity was determined in the above-mentioned tests carried out with human volunteers. This activity was determined during the last days preceding the period of consumption of yogurt prepared, for example, with the CNCM I-1225 strain, throughout that period and for the first few days following it.

It was found that this activity changed from 8.2 to 4.9 μg/h/mg protein during the period of consumption of the yogurt, remained at that level for about one week after that period and then increased progressively increased.

Immunomodulation

Human volunteers (phagoytic power of leucocytes)

Human volunteers abstained from eating fermented milk products except for the products eaten in accordance with the following program: milk for three weeks, yogurt prepared by fermentation of a milk with a mixed culture of commercial *S. thermophilus* and *L. acidophilus* CNCM I-1225, for example, for the following three weeks and then milk for six weeks.

The phagocytic power of the leucocytes in the peripheral blood of the volunteers was determined at the beginning and at the end of each of these periods.

This determination comprises extracting the leucocytes from the blood and bringing them into contact with fluorescent bacteria. The fluorescent light emitted by the leucocytes which had phagocytosed the fluorescent bacteria was measured by cytometric analysis in flux (using an apparatus of the type commercially available under the name of Facscan). The percentage of leucocytes showing phagocytic activity, i.e. the phagocytic power mentioned above, was deduced therefrom.

A phagocytic power of the leucocytes in the peripheral blood of 36.5% was observed at the beginning of the first period of consumption of milk 32.7% at the end of that period and hence at the beginning of the period of consumption of yogurt, 51.8% at the end of the period of consump-

tion of yogurt and 51.4% six weeks afterwards, i.e. at the end of the second and last period of consumption of milk. The probability of an error being made (p value) by estimating that this increase in the phagocytic power of the leucocytes is significant is less than 0.1%.

Human volunteers (response to a vaccine)

16 Healthy human volunteers (test group) followed the following eating program: for two weeks (weeks 1 and 2), normal diet excluding any fermented product; for the following three weeks (weeks 3, 4 and 5), mixed diet of three 125 ml yohurts per day, the yogurts having been prepared by fermentation of a milk with a commercial culture of *S. thermophilus* and *Bifidobacterium bifidus* to which the *L. johnsonii* strain CNCM I-1225—present in this yogurt—was added in a quantity of 10^7 to 10^8 cfu/ml; and for another two weeks (weeks 6 and 7) normal diet excluding any fermented products.

14 Healthy human volunteers (control group) simultaneously followed an eating program consisting of a normal diet excluding any fermented product.

A vivotive oral vaccine (*Salmonella typhi* Ty21a) marketed by Berna SA was administered to the volunteers of the two groups in accordance with the manufacturer's instructions on days 1, 3 and 5 of week 4.

Blood samples were taken from all the volunteers 3 days after the beginning of week 3 and 1 day and 10 days after the end of week 5.

Determination of the concentration of the specific IgA's of the immune response to the antigenic lipopolysaccharides (LPS) of *Salmonella typhi* was carried out by the ELISA method.

It was found that the increase in the concentration of the specific IgA's observed fifteen days after vaccination in relation to the concentration observed nine days before vaccination is significant in the two groups (p value >0.001).

However, if ranges of increase factors <2; >2 and <3; >3 and <4; >4 are taken into consideration, respective distributions are observed in these ranges of 1, 6, 3 and 6 volunteers for the test group against 8, 3, 0 and 3 volunteers for the control group. In other words, the increase factors are significantly higher in the test group than in the control group (p value=0.04).

Adhesion to intestinal cells

According to the invention, a study was made of the adhesion of various strains of lactic acid bacteria to intestinal cells, more particularly to Caco-2 human epithelial intestinal cells (M. Pinto et al., Biol. Cell. 47, 323, 1983) and to mucus-secreting human intestinal cells HT29-MTX (Lesuffleur et al., Cancer Res. 50, 6334–6343) in a monolayer culture in vitro.

To this end, the cells were cultured in 25 cm^2 plastic bottles (Corning) for maintaining the cell lines and on degreased and sterilized glass slips (22×22 mm) placed in 6-cup trays (Corning) for the adhesion tests.

To cultivate the Caco-2 and HT29-MTX cells, the medium had to be changed daily from the second day after reseeding. The culture medium was prepared from Eagle minimum essential medium powder modified with Dulbecco (DMEM).

The lactic bacteria were cultured in anaerobiosis on MRS medium from a frozen stock. The bacteria were used from the second subculture.

A mixed medium for incubation on the cells was prepared by mixing 50% of a DMEM medium without antibiotic and 50% of the MRS medium in which the bacteria had grown, this medium containing 10^8 lactobacilli or bifido bacteria (cf. Chauvière G. et al., FEMS Microbiol. Lett. 91, 213–218, 1992).

To carry out the adhesion test, the mixed medium containing the bacteria was placed on the intestinal cells and incubated for one hour in aerobiosis. The multiple-cup trays were washed five times by twenty circular agitations to enable the non-adhering bacteria to be effectively eliminated. The cell lawns were then fixed in successive baths of methanol, 10 mins. at 70%, 10 mins. at 95% and 15 mins. at 100%, and coloured with Gram or Giemsa coloration. An adhesion level was determined by counting the adhering bacteria under a microscope.

Among the numerous strains tested, the four strains lodged by way of example for the purposes of the present invention showed a good level of adhesion to intestinal cells as determined by these adhesion tests on the Caco-2 cell line.

Thus, the *L.johnsonii* strain CNCM I-1225 adhered to the Caco-2 cells to a level of approximately 150+23 bacterium cells per 100 Caco-3 cells. If this result is given a score of +++++, an adhesion of +++++ is obtained for *B. breve* CNCM I-1226, a score of ++++ for *B. infantis* CNCM I-1227 and a score of ++++ for *B. longum* CNCM I-1228.

Tests to determine the adhesion of the *L-johnsonii* strain CNCM I-1225, for example, to the HT29-MTX cells produced even more spectacular results.

It was also surprisingly found that the adhesion of these strains or at least some of them was due to a factor which they secrete in their own culture medium (MRS or milk for example). Thus, when the process of incubation for 1 hour on Caco-2 described above was carried out with the strains *L. johnsonii* CNCM I-1225, *B. breve* CNCM I-1226 or *B. longum* CCNCM I-1228 without their bacterial culture medium, a significant reduction in adhesion was observed.

In addition, when this process of incubation was carried out on Caco-2 with these strains and their culture medium subjected beforehand to treatment with trypsin, a significant reduction in adhesion was again observed. This would appear to prove that the adhesion factor secreted by these strains in their culture medium is a protein.

Competitive exclusion of pathogenic bacteria

According to the present invention, a study was made of the various lactic acid bacterium strains for their capacity for competitive exclusion of pathogenic bacteria, more particularly the pathogenic bacteria responsible for diarrhoea, from intestinal cells.

In particular, a study was made of the exclusion of certain saprophytic strains of *E. coli* from the digestive tube of human beings and animals which can assume a virulent character and can become pathogenic, namely enterotoxinogenic *E. coli* (ETEC), enteroadherent *E. coli* (DAEC) and enteropathogenic *E. coli* (EPEC), and of the exclusion of a strain of *Salmonella typhi-murium*.

The strains used for this study are as follows:

- for ETEC, the strain H10407 which expresses CFA/1 (Collection of Professeur Joly, Laboratoire de Microbiologie, Faculté de Médecine et de Pharmacie, Université de Clermont-Ferrand 1, 63003 Clermont-Ferrand, France)
- for DAEC, the strain C1845 (collection of Dr. S. Bilge, Department of Microbiology, School of Medicine, G 3111 Health Sciences Building, University of Washington, Seattle, Wash. 98195, USA)
- for EPEC, the strain JPN15 pMAR7 which expresses BAF and eae (collection of Prof. J. Kaper, Center for Vaccine Development, University of Maryland, School of Medicine, 10 South Pine Street, Baltimore, Maryland 21201, USA)
- for *Salmonella typhi-murium*, the strain SL 1344 (Dr. B. Stocker, Stanford University, School of Medicine,

Department of Microbiology and Immunology, Sherman Sairchild Science Building, D 333 Stanford, Calif. 94305–5402, USA).

The adhesion of the bacteria to the Caco-2 cells was determined as follows:

Briefly, the monolayers of Caco-2 cells were washed twice with a saline phosphate buffer (PBS). The ^{14}C-labelled *E. coli* or the ^{35}S-labelled *Salmonella* were suspended in the culture medium in a quantity of 10^8 cfu/ml and 2 ml suspension were added to each cup containing a slip bearing the cell culture.

For *E. coli*, the incubations were all carried out in the presence of 1% D-mannose. To determine an exclusion factor or level, i.e. the proportion of pathogenic bacteria prevented from adhering to the Caco-2 cells by lactic bacteria which take their place, 1 ml suspension containing 10^8 cfu/ml labelled pathogenic strain and 1 ml of a suspension containing either 10^8 or 10^9 cfu/ml of the lactic acid bacterium strain tested were added to each cup containing a slip bearing the cell culture.

The plates are incubated for 1 hour at 37° C. in an atmosphere of 10% CO_2 and 90% air. The monolayers of cells are washed 5 times with sterile PBS. The adhering bacteria and the intestinal cells are dissolved in 0.2 N NaOH solution. The number of labelled adhering bacteria is evaluated by liquid scintillation counting.

Among the various strains of lactic bacteria thus tested for their performance properties or for their capacity for competitive exclusion of pathogenic bacteria, the strains selected and lodged by way of example for the purposes of the present invention produced remarkable results as set out in the following Table which shows in % the exclusion levels achieved by the strains tested at the expense of the various pathogenic strains used in these tests.

Strain (CNCM No.)	Concentration (cfu/ml)	Competitive exclusion factor (%) with respect to: ETEC	DAEC	EPEC	Salmonella
I-1225	10^9	78	79	83	86
	10^8	50	53	53	42
I-1226	10^9	80	68	83	88
	10^8	55	53	55	41
I-1228	10^9	47	47		
	10^8	11			
I01227	10^9	58	46		
	10^8	18			

We claim:

1. A biologically pure culture of *Lactobacillus johnsonii* strain CNCM I-1225.

2. A cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus johnsonii* strain CNCM I-1225.

3. A food composition comprising an ingestible support and a culture of *Lactobacillus johnsonii* strain CNCM I-1225.

4. A food composition according to claim **3** wherein the ingestible support is an acidified milk product.

5. A food composition according to claim **3** wherein the ingestible support is a yogurt.

6. A food composition according to claim **3** wherein the ingestible support is a milk-based powder.

7. A food composition comprising an ingestible support and a cell-free culture supernatant isolated from a biologically pure culture of *Lactobacillus johnsonii* strain CNCM I-1225.

8. A food composition according to claim **7** wherein the ingestible support is an acidified milk product.

9. A food composition according to claim **7** wherein the ingestible support is a yogurt.

10. A food composition according to claim **7** wherein the ingestible support is a milk-based powder.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : **5,603,930**

DATED : **February 18, 1997**

INVENTOR(S) : **Dominique Brassart, et al.**

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, under the heading "[56] References Cited, Foreign Patent Documents",

delete "05849 6/1989 European Pat. Off. .
09608 7/1991 European Pat. Off. ."

and insert therefor

—WO 05849 6/1989 WIPO.
WO 09608 7/1991 WIPO.—.

Col. 4, line 48, change "acidophilus" to —johnsonii—.

Signed and Sealed this

Twenty-sixth Day of August, 1997

Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

EXHIBIT H

United States Patent [19]

Reddy

[11] **Patent Number: 4,806,368**

[45] **Date of Patent: Feb. 21, 1989**

[54] **SHELF LIFE AND SUBSEQUENT GROWTH OF *LACTOBACILLUS ACIDOPHILUS, PROPIONIBACTERIUM SHERMANII* AND *LEUCONOSTOC CITROVORUM* IN DIETARY FIBER BASED SUPPLEMENT PREPARATION**

[76] Inventor: **Malireddy S. Reddy,** 6983 S. Telluride St., Aurora, Colo. 80016

[21] Appl. No.: **97,061**

[22] Filed: **Sep. 16, 1987**

[51] **Int. Cl.**[4] **A23C 9/12**

[52] **U.S. Cl.** **426/61;** 426/71; 426/72; 426/73; 426/74; 424/464; 424/465; 424/441

[58] **Field of Search** 424/464, 465, 441; 426/72-74, 61, 71

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Primary Examiner—Jacqueline V. Howard
Assistant Examiner—Andrew Griffis
Attorney, Agent, or Firm—Kyle W. Rost

[57] **ABSTRACT**

Dietary fiber based tablets with *Lactobacillus acidophilus* and/or *Bifidobacterium bifidus, Leuconostoc citrovorum* and *Propionibacterium shermanii,* are prepared. To enhance the viability of *L. acidophilus* in the tablets, a combination of amino acid L-cystine, vitamin-C and vitamin-E are included. To protect and enhance the beneficial bacterial population in the human stomach and intestinal tract, calcium and magnesium salts have been incorporated along with the dietary fiber in the formula. Lecithin has been used as a lubricant. The minor amount of lactose sugar has been incorporated into the formula using preparations of acid whey, whey protein concentrate, and pure lactose. The proposed formula when tableted gave maximum protection to the *L. acidophilus* population, in comparison to the free flowing powder form. Vitamin A and D are included in the formula as nutritional supplements. Autolyzed yeast extract and enzyme digested casein have been incorporated as stimulants to the beneficial bacteria. In addition, autolyzed yeast extract serves as a major source of B-vitamins.

19 Claims, No Drawings

SHELF LIFE AND SUBSEQUENT GROWTH OF *LACTOBACILLUS ACIDOPHILUS, PROPIONIBACTERIUM SHERMANII* AND *LEUCONOSTOC CITROVORUM* IN DIETARY FIBER BASED SUPPLEMENT PREPARATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to the development of a nutritional supplement (in the form of a tablet) using a lyophilized preparation of *Lactobacillus acidophilus, Propionibacterium shermanii,* and *Leuconostoc citrovorum* along with a major proportion of dietary apple fiber, lecithin, L-cystine, and mineral supplements of the origin calcium and magnesium. The invention permits the longevity of the health promoting bacteria in the tablets. Also, the survival and growth of these bacteria in a simulated human gastrointestinal system is greatly enhanced using the proposed invention.

2. Description of the Prior Art

Lactobacillus acidophilus is claimed to have therapeutic value in human beings. The organism is ingested in the form of acidophilus milk or sweet acidophilus milk or as a tablet form along with lactose sugar. In health food stores, *Lactobacillus acidophilus* has been sold in the form of tablets with the following composition: *L. acidophilus,* lactose, carboxymethyl cellulose, etc. As a matter of fact, this is the most convenient form to ingest these organisms. Some companies also sell *L. acidophilus* in the form of powder. The powder is supposed to be added to milk and then consumed. In one instance *L. acidophilus* has also been mixed with dietary fiber and sold not as tablets but in a powder form. The major problem with the available technology is that *L. acidophilus* is not viable for a great length of time. The 1983 University of Wyoming survey conducted by Brennan et. al. proved that the viability claims of manufacturers do not hold true with these products. Also, a majority of the injured or non injured (but low concentration) *L. acidophilus* bacterial cells get rapidly inactivated in the human stomach, where the pH is close to 2.0 to 2.9.

In recent years, yeast related problems are increasing dramatically. This is especially found in the intestinal tract and female reproductive organs. It is a long known fact that propionic acid inhibits yeast and molds. Propionic acid is produced by harmless *Propionibacterium shermanii* or by several species of *Propionibacterium* by using either glucose, lactose, or lactic acid as a substrate. The *Propionibacterium* can thus reduce lactate or lactic acid to propionic acid and carbon dioxide, which can retard the growth of yeasts and molds. In addition, propionic acid bacterium can synthesize B-vitamins in the human gastrointestinal tract.

It has also been stated in the literature that due to the growth of certain yeasts in the human gastrointestinal tract, a compound called acetaldehyde can accumulate and later get into the blood circulation. Scientists claim that this compound, acetaldehyde, has a pronounced depressing effect in human beings. *Leuconostoc citrovorum,* a beneficial organism that is used in the manufacture of buttermilk, has an enzyme called alcohol dehydrogenase which can destroy the acetaldehyde.

It has been clearly stated in the literature that *Lactobacillus acidophilus* has the following health benefits: 1. reduction of colon cancer, 2. reduction of cholesterol uptake into the blood stream, 3. reduction of lactose intolerace, 4. reduction of intestinal flatulence due to the growth of putrefactive bacteria.

It has been claimed in the literature that diet fiber or food fiber when consumed in recommended quantities will reduce: 1. constipation, 2. intestinal diverticulosis, 3. excess cholesterol and, 4. spastic colon. There have also been claims in the literature regarding the cancer retardation properties of vitamins E and C.

SUMMARY OF THE INVENTION

The primary object of the invention is to enhance the viability of *Lactobacillus acidophilus* in tablets and also in the gastrointestinal tract.

It is another object to include and extend the viability of *Propionibacterium shermanii* and *Leuconostoc citrovorum* along with *L. acidophilus* by the proposed tablet composition.

Another object is to protect and enhance the growth of *L. acidophilus* in the gastrointestinal tract by adding apple dietary fiber to the tablets. Furthermore, the dietary fiber may have healthful benefits such as tending to reduce colon cancer and decreasing the serum cholesterol level.

It is another object to replenish some of the minerals and vitamins that are lost due to the ingestion of fiber, as fiber speeds up the evacuation of bowel contents.

As far as is known, none of the tablets currently available in the market have *Propionibacterium shermanii* or species of Propionibacterium, *Leuconostoc citrovorum* along with *Lactobacillus acidophilus.* Also, the reducing compounds such as L-cystine have not been included in *acidophilus* tablets to enhance viability over a long period of time.

According to the invention, lyophilized bacterial cultures (*L. acidophilus, P. shermanii,* and *L. citrovorum*), reducing compounds, vitamins, minerals, lecithin, milk derived nutrients, autolyzed yeast extract and apple fiber are mixed together to make the tablets. The reducing compounds such as L-cystine are used to enhance the viability of the lyophilized bacterial preparations in the tablets. The vitamins such as vitamin E, vitamin C are employed as nutritional supplements. The minerals such as calcium carbonate and magnesium oxide are used as mineral supplements. The lecithin is employed as a natural lubricant to aid the tablet preparation. The milk derived ingredients such as acid whey powder, whey protein concentrate, and enzyme digested casein are used as bacterial growth enhancers. Also, the acid whey supplies bio-available minerals, simple and complex carbohydrate substrates, and lactates to the bacterial growth in the intestinal tract. Further, autolyzed yeast extract has been included in the tablets not only to stimulate bacterial growth in the gastrointestinal tract, but also as a B vitamin supplement. In addition, the autolyzed yeast extract also will supply most of the major and minor trace minerals. These stimulants, minerals, lactose, and vitamin D have been included in the current tablets to obviate some of the negative aspects of the dietary fiber. It has been reported that vitamin D and lactose have a major role in the absorption of calcium in the human gastrointestinal tract. The autolyzed yeast extract is an intracellular yeast substance obtained by breaking the yeast cells. In essence, the live yeast cells do not exist in the autolyzed yeast extract preparation. Even though lactose sugar helps the growth of *L. acidophilus* in the intestinal tract, several people are allergic to lactose sugar due to lactose intolerance.

However, in the present formulation, the lactose content is significantly decreased to eliminate this discomfort. Consequently these tablets can be consumed even by the lactose intolerant individuals and still derive the healthful benefits of *Lactobacillus acidophilus.*

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Two types of the tablet formulations are developed. One is designated "regular" and the other set is "diet" type. The composition of these tablets (750 mg) are as follows:

TABLET COMPOSITION AND METHOD OF PRODUCTION

Ingredients	Regular Type	Diet Type
Apple Fiber	320 mg	510 mg
L-cystine	3 mg	1 mg
Lecithin (powdered)	20 mg	20 mg
Acidophilus powder	100 mg	25 mg
Propionic Acid Bacterium Powder	2.5 mg	0.5 mg
Leuconostoc citrovorum Powder	2.5 mg	0.5 mg
Autolyzed Yeast Extract	40 mg	15 mg
Acid Whey Powder	50 mg	30 mg
Whey Protein Concentrate	10 mg	2.5 mg
Vitamin - A	1500 I.U.	425 I.U.
Vitamin - D3	150 I.U.	42.5 I.U.
Vitamin - E	10 I.U.	2 I.U.
Vitamin - C	20 mg	10 mg
Calcium Carbonate	90 mg	75 mg
Magnesium Carbonate	5 mg	5 mg
Magnesium Oxide	5 mg	5 mg
Calcium Phosphate	20 mg	10 mg
Lactose	45.5 mg	32 mg
Enzyme Digested Casein	4 mg	5 mg

These ingredients were combined according to the following method. The acidophilus powder, propionic acid bacterium powder, Leuconostoc powder, acid whey, whey protein concentrate, autolyzed yeast extract, enzyme digested casein and calcium phosphate should be fine and they should pass through a 35 mesh (U.S. No. 40 Screen—Tyler). If they are not fine, they should be gently ground to meet the specification. Then L-cystine, acidophilus powder, propionic acid bacterium powder, Leuconostoc powder, acid whey, whey protein concentrate, vitamin A, vitamin D3, vitamin E, vitamin C, calcium carbonate, magnesium carbonate, lactose, calcium phosphate, magnesium oxide, autolyzed yeast extract and enzyme digested casein are mixed with an equal quantity of apple or any other vegetable dietary fiber. Particular care should be given to the magnesium carbonate and calcium carbonate to adequately distribute them throughout the mix. Then the above prepared ingredient mix is combined with the reminder of the dietary fiber. The ingredients are mixed thoroughly and then the powdered lecithin is added. The total mix is passed through a 20 mesh screen, remixed and passed again through the 20 mesh screen. Then the mix is tableted using a single punch Colton 330–33 machine. The punch used was one-half inch standard concave with a fill weight adjusted to 750 mg (+5%).

The tablets were compressed to a hardness of 11 to 14 kg (Pfizer). The thickness of the tablets varies up to 5.9 mm(1 to 2%) (Ames). After forming, the tablets were stored in metal containers.

DESCRIPTION

The regular version of the tablets (750 mg) will supply the high concentrations of *L. acidophilus* and other bacteria if they are consumed at the rate of 3 per day. Three regular tablets per day will supply the U.S.R.-D.A. vitamin A, D, E,and C requirements. The diet version is designed to supply the maximum amount of dietary fiber. Since these tablets have to be consumed in large quantities, the vitamin, mineral, and bacterial concentrations have been significantly reduced. Using the same ratio of ingredients, tablets can be made of any size ranging from 250 mg to 2500 mg.

According to one novel aspect of the invention, L-cystine, vitamin E, and vitamin C, when incorporated into the tablets, produce the exceptional result of enhancing the viability of the *Lactobacillus acidophilus.* The primary intent of using these vitamins in these tablets is to supply the vitamins along with acidophilus. Later it was discovered that they protect the bacterial cells in the tablets, apparently due to their antioxidation properties. However, L-cystine has been deliberately added to reduce the oxygen in the tablets so that the bacterial cell life can be extended. Experimentally, it is proven in this invention that a combination of L-cystine, vitamin C, and vitamin E had a pronounced effect on maintaining the viability of the bacterial cells in the presence of dietary fiber and other ingredients. It should be noted that dietary fiber of the type employed contains insoluble fiber elements.

It has been stated in the literature that dietary fiber has an exceptional ability of imbibing water and thus moves food faster in the human gastrointestinal tract. Consequently, it can decrease the mineral uptake by the intestinal mucosa. To offset this, I have included calcium and magnesium carbonates, oxides, and phosphates. Later, it was discovered that these mineral salts are extremely essential for the survival of the *Lactobacillus acidophilus* in the low pH stomach contents.

Also, the diet fiber is included in the tablets as a dietary supplement. A further aspect of the invention is the discovery that after reconstitution, the diet fiber (apple) has an exceptionally protective and stimulatory effect on the growth of *Lactobacillus acidophilus,* in the presence of neutralizing calcium and magnesium salts.

Thus this invention is novel not only to protect the viability of the therapeutic bacteria in the tablets but also to protect them under the acidic conditions of the human stomach. In addition, the formulation has a positive effect of stimulating the growth of *L. acidophilus* in the presence of bile salts, which condition exists in the human small intestine.

The harmless Swiss cheese associated propionic acid bacterium is included along with the *L. acidophilus* for the following reasons:

1. Propionic acid bacterium can create slightly anaerobic conditions which is conducive for the growth of *L. acidophilus.*

2. The excess lactic acid produced by *L. acidophilus* in the gut can be converted by propionic acid bacterium to propionic acid and carbon dioxide. The propionic acid has a pronounced effect on retarding mold and yeast growth.

3. The propionic acid bacteria can synthesize B vitamins, which can further stimulate *L. acidophilus.*

4. The propionic acid bacteria have catalase enzyme so that they can detoxify hydrogen peroxide to water

and oxygen. Hydrogen peroxide is highly detrimental to the growth *Lactobacillus acidophilus.*

The *Leuconostoc citrovorum*, a micro organism which is used in the manufacture of buttermilk, is included in the tablets along with the *L. acidophilus* and propionic acid bacterium for the following reasons:

1. *Leuconostoc citrovorum* has an enzyme alcohol dehydrogenase, which can destroy acetaldehyde. It has been claimed that the growth of yeasts in the intestinal tract produces acetaldehyde, which is responsible for depression symptoms in human beings.

2. This organism can utilize simple sugars and citrate and thus can produce a slight amount of carbon dioxide, which is beneficial for the growth of *L. acidophilus* and propionic acid bacterium.

Even though several therapeutic benefits exist due to these bacteria, the real problem has been how to keep them active and have them grow and attach to the human intestinal tract. This invention can achieve this goal. Both the regular and diet type tablets have been tried on an experimental basis on several age group humans and there were no side effects, such as excessive gas, variations in stools etc. The single most positive comment given was the dramatic improvement of the bowel movement. Also, the diet tablets greatly improved the stamina of people who are on 1000 calorie/-day diet, besides helping them to loose weight and avoid the bowel problem.

The method of present invention is further illustrated by the following examples:

EXAMPLE 1

The ingredients used in this and all other examples were obtained from the following sources:

1. Apple fiber—Tree Top, Inc., P.O. Box 248, Selah, Wash. 98942.

2. *Lactobacillus acidophilus* lyophilized preparations—Chris Hansen Laboratories, 9015 West Maple St. Milwaukee, Wis. 53214

3. Lyophilized Propionibacterium and Leuconostoc cultures—Vivolae Cultures Corp., 3862 East Washington St., Indianapolis, Ind. 46201

4. L-cystine—Nutritional Bio-chemicals, Cleveland, Ohio

5. Calcium phosphate—Strauffer Chemical Co. Food Ingredient Div. Cheeseburough Ponds Inc., West Port, Conn. 06881-0852

6. Calcium Carbonate—Pfizer, Inc.; Minerals, Pigments, and Metals Division; New York, N.Y. 10017

7. Magnesium oxide and magnesium carbonate, and vitamin C—Merck and Co., Rahway, N.J.

8. Vitamin A, D, E—Roche Chemical Division, Hoffman Laroch Inc., Nutty, N.J. 07110

9. Acid whey powder—Deltown, Chemurgic Corp., 170 Sawmill River Rd., Yonkers, N.Y. 10701

10. Whey protein concentrate, lactose and enzyme digested casein—Mid America Dairymen Inc., Springfield, Mo. 65805-1837.

11. Autolyzed yeast extract—Busch Industrial Products Corporation, St. Louis, Mo. 63127.

12. Lecithin—American Lecithin Company, P.O. Box 4036, Atlanta, Ga. 30302.

The bacteria powders employed contain lyophilized bacteria, which remain viable. By the term, "lyophilized," it is intended to include substantially any technique or variation for preserving viable bacteria in a dry or powdered form, such that the bacteria can be incorporated into a tablet formulation.

The regular type tablets were prepared according to the composition outlined under the section entitled TABLET COMPOSITION AND METHOD OF PRODUCTION. In one set of the samples, calcium carbonate, magnesium carbonate, magnesium oxide and calcium phosphate were eliminated. In an another set of samples only fiber was eliminated. These individual tablets were dissolved in 15.0% solids reconstituted whey protein concentrate (31% protein) whose pH has been adjusted to 2.8 using hydrochloric acid. In this example, the whey protein concentrate was used to simulate food and pH was lowered to 2.8 to simulate the acidic conditions of the human stomach. In each one hundred ml of the simulated human stomach contents, 1 tablet (750 mg) was dissolved. The three tablets, representing three different compositions, were dissolved in 3 different bottles. The initial concentration of *Lactobacillus acidophilus* was determined by plating on MRS Agar with 0.15% bile salts. Then the three bottles were incubated at 37° C. (human body temperature) for 4h. This 4h Incubation time was elected to simulate the retention time of food in human stomach. At the end of the incubation, the *L. acidophilus* counts again were determined. Then to each bottle, 0.15% of bile salts were added and the pH of the contents were adjusted to 7.1+0.1. This part will simulate the human intestinal tract. Then the bottles were incubated for 4 more hours at 37° C. At the end of this second incubation, the total *L. acidophilus* counts again were determined. The results are summarized in Table I. These results indicate that, when calcium and/or magnesium salts are not included in the tablets, the viability of *L. acidophilus* was reduced significantly and the organisms didO not recover even when the pH was raised to 7.0. On the contrary, when the diet fiber was eliminated from the tablets, there was only a slight reduction in the numbers of *L. acidophilus* in the stomach. However, there was no apparent increase of the growth of *L. acidophilus* upon further incubation at pH 7.0. The third tablet, in which all the ingredients were included (calcium and/or magnesium salts plus the diet fiber), there was from an insignificant reduction to no reduction in the viability of the *L. acidophilus* during the first four hour incubation (simulated stomach conditions). In addition, there was a significant increase of the growth of *L. acidophilus* observed after pH was raised to 7.0 and then incubated for 4 more hours. This clearly proves that the calcium and magnesium salts have a pronounced effect of protecting the *L. acidophilus* bacteria under the acidic conditions that exist in the stomach. This is partly due to their neutralizing effect on the stomach acids. Diet fiber alone could not protect the *L. acidophilus* from acid damage. Both the fiber and calcium and/or magnesium salts have a synergistic effect of not only protecting the *L. acidophilus* bacteria from acid injury, which is incurred under the stomach conditions, but also stimulating these bacteria to grow under the conditions prevalent in the small and large intestinal tract. This is a very novel aspect of this invention. Similar results were obtained with the diet formula of the current invention with both *L. acidophilus* and/or *Bifidobacterium bifidus.*

TABLE I

No.	Variables in the tablet formulation	*Lactobacillus acidophilus* Counts/gram at: 0 Time	After 4 h Incubation at 37 C. at pH 2.8	After 4 h Additional Incubation at 37 C. at pH 7.0 in the presence of 0.15% Bile Salts
1	Fiber but no Calcium and Mg Salts added	80×10^5	100×10^1	60×10^1
2	No Fiber but Ca and Mg Salts added	80×10^5	40×10^5	60×10^5
3	Fiber plus Ca and Mg Salts added	80×10^5	60×10^5	200×10^6

EXAMPLE 2

The effect of stimulants upon the survival and proliferation of *Lactobacillus acidophilus* has been determined. The tablets were prepared using the regular formula, except for the following variations:

1. The first batch was prepared with all the ingredients, including three stimulants: autolyzed yeast extract, acid whey, and enzyme hydrolyzed casein—each 32 mg/750 mg tablet.

2. The second batch was prepared using only the yeast extract as stimulant—96 mg/750 mg tablet.

3. The third batch was prepared using only the acid whey as stimulant—96 mg/750 mg tablet.

4. The fourth batch was prepared using only the enzyme digested casein—96 mg/750 mg tablet.

5. The fifth batch was prepared with no stimulants. In this case, the amount of stimulant was replaced by the apple fiber to arrive at the equal weight.

The experimental procedure was same as that outlined in Example 1.

The results of this example are presented in Table 2. From these it appears that all three stimulants have a pronounced effect on the growth of *L. acidophilus* in the presence of bile salts. In order of preference, the autolyzed yeast extract ranked number one, followed by enzyme digested casein and acid whey. However, when all three stimulants were used, there was a significant increase of *L. acidophilus* over the use of an individual stimulant. This could be due to some interactive effect.

TABLE II

No.	Variable in the tablet formulation	*Lactobacillus acidophilus* Counts/gram at: 0 Time	After 4 h Incubation at 37 C. at pH 2.8	After 4 h Incubation at pH 7.0 in the presence of 0.15% Bile Salts
1	All three stimulants included	68×10^5	53×10^5	280×10^6
2	Only autolyzed yeast extract included	68×10^5	50×10^5	180×10^6
3	Only acid whey included	68×10^5	42×10^5	82×10^6
4	Only enzyme digested casein included	68×10^5	57×10^5	150×10^6
5	None of the stimulants included	68×10^5	45×10^5	100×10^5

EXAMPLE 3

To determine the effect of reducing amino acid L-cystine on the survival of *Lactobacillus acidophilus,* the following example is illustrated. The regular type tablets were prepared using the formula outlined above in TABLET COMPOSITION AND METHOD OF PRODUCTION. As a variation, in one batch, the amino acid L-cystine was eliminated. Since the antioxidants vitamin-C and vitamin-E are in the formulation, their effect on the viability of *L. acidophilus* also was evaluated. To accomplish this, in one formulation, both the vitamin-C and vitamin-E were eliminated. As a negative control, in one batch of tablets, L-cystine and the vitamins C and E were completely eliminated. In all these four batches of tablets, the initial concentration of *L. acidophilus* was determined at the time of tableting. Then the tablets were stored for 3 months at refrigeration temperature. At the end of the storage period, the *L. acidophilus* counts were once again determined. The results are presented in Table 3. From these data, it is obvious that the elimination of L-cystine and vitamins C and E from the formulation, resulted in a significant decrease in the viability of *L. acidophilus* during storage. The inclusion of vitamin C and E alone without L-cystine, restored partially the viability of *L. acidophilus.* However, L-cystine alone, without the vitamins C and E, was far superior in retaining the viability of *L. acidophilus* in the current formulation. It is very interesting to observe that the combination of L-cystine, vitamin-C and vitamin-E has significantly increased the viability of *L. acidophilus* in the current formulation.

The vitamins were included as nutritional supplements. Surprisingly it turned out that the combination of L-cystine and vitamins C and E greatly improved the viability of *L. acidophilus* over the amino acid L-cystine alone. This is an other significant feature of this invention.

TABLE III

No.	Variable	*Lactobacillus acidophilus* Count/tablet at: 0 Time	After three months storage
1	No L-cystine but ascorbic acid and vitamin E are added	200 × 10^6	20 × 10^6
2	L-cystine but no ascorbic acid and vitamin E are added	200 × 10^6	80 × 10^6
3	L-cystine plus ascorbic acid and vitamin E are added	200 × 10^6	130 × 10^6
4	No L-cystine, no ascorbic acid and no vitamin E are added	200 × 10^6	60 × 10^5

EXAMPLE 4

Using the formula described in TABLET COMPOSITION AND METHOD OF PRODUCTION, an experiment was designed to check the effect of tableting on the viability of *Lactobacillus acidophilus* upon storage. After all the powders have been mixed, one half of the sample was further processed to make tablets. The other half was allowed to stay in the powder form. The initial population of *L. acidophilus* was determined in both the preparations. Then the samples were stored for 3 months, part at 4° C. and part at room temperature (21° C.). At the end of the storage period, the samples—both the powder and tablet form of the formulation—were plated to determine the total count of *L. acidophilus* bacteria. The results of this example are presented in Table 4. It is apparent from the data that the proposed formula prolonged the viability of *L. acidophilus* when tableted rather then in powder form. This result is logically explained by the exclusion of the air from the formulation by tableting, since air is toxic to *L. acidophilus* during storage. Even though the antioxidants are included in the formula, it is necessary to exclude the entry of air by tableting for the improved viability of *L. acidophilus* and other beneficial bacteria. Also, the present formulation can extend the shelf life of *L. acidophilus* even when the tablets are stored at room temperature.

TABLE IV

No.	Variable	*Lactobacillus acidophilus* Count/tablet at: 0 Time	After three months storage
1	All the ingredients are pulverized and left as loose powder	160 × 10^6	40 × 10^6
2	All the ingredients are pulverized and compressed as tablets	160 × 10^6	110 × 10^6

EXAMPLE 5

The effectiveness of the current formulation in maintaining the viability of *Propionibacterium shermanii* and *Leuconostoc citrovorum* was investigated. The tablet formulation and the amounts of lyophilized cultures used were same as outlined under the section entitled TABLET COMPOSITION AND METHOD OF PRODUCTION. All three bacterial preparations were included. After the tablets were made (regular type), the initial concentrations of *Propionibacterium shermanii* and *Leuconostoc citrovorum* were determined using special selective agars. Then the tablets were tightly sealed and stored for a period of 3 months at 4° C. At the end of the storage period, once again the *Propionibacterium shermanii* and *Leuconostoc citrovorum* counts were determined. The results of this study are presented in Table 5. The data clearly indicate that the present formulation has a tremendous protective effect upon the *Propionibacterium* and *Leuconostoc* in addition to the *Lactobacillus acidophilus.*

TABLE V

No.	Bacterial strain Designation	Bacterial counts/tablet obtained at: "0" Time	After 3 months storage
1	*Propionibacterium shermanii*	200 × 10^4	110 × 10^4
2	*Leuconostoc citrovorum*	160 × 10^4	92 × 10^4

EXAMPLE 6

The efficiency of the present formulation upon maintaining the viability of the following beneficial bacterial species was investigated: *Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus caucasicus, Lactobacillus lactis, Lactobacillus helveticus, Streptococcus lactis, Streptococcus cremoris, Streptococcus diacitlactis, Streptococcus durans, and Streptococcus faecalis.* The tablet formulation was same as in example 5, except lyophilized preparations of the above specified organisms along with *L. acidophilus, B. bifidus, P. shermanii,* and *L. citrovorum* were included at the rate of 7 mg of each organism per 750 mg tablet. Since all the fifteen organisms were included, the total amount of bacterial preparations came to 105 mg/750 mg tablet. The total bacterial counts and the Enterococcus counts were determined at "0" time and after 3 months storage at 4° C. Even though accurate counts of each organism were not determined because of the complexity, on the basis of the total counts and Enterococcus counts, it appears that these organisms are protected in the tablets with the present formulation. The results of this study are presented in Table 6.

TABLE VI

Bacterial counts/tablet obtained at:					
32 C. Using tryptic soy agar		37 C. Using tryptic soy agar		37 C. using KF agar	
"0" Time	After 3 Mo.	"0" Time	After 3 Mo.	"0" Time	After 3 Mo.
68×10^6	21×10^6	43×10^6	120×10^5	120×10^5	100×10^5

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact formulation and method shown and described, and accordingly all suitable modifications and equivalents may be regarded as falling within the scope of the invention as defined by the claims that follow.

I claim:

1. A dietary fiber based vitamin, mineral, and beneficial bacteria tablet with enhanced bacterial viability, comprising:

apple fiber including insoluble fiber elements;

lyophilized live bacteria;

a vitamin having antioxidant properties;

an amino acid having reducing properties;

an alkaline mineral salt; and

means for stimulating bacterial growth.

2. The tablet of claim **1**, wherein said lyophilized live bacteria is selected from the group consisting of *Lactobacillus acidophilus, Bifidobacterium bifidus, Propionibacterium shermanii, Leuconostoc citrovorum, Streptococcus thermophilus, Lactobacillus bulgaricus, Streptococcus lactis, Streptococcus cermoris, Streptococcus durans, Streptococcus faecalis, Lactobacillus casei, Lactobacillus caucasicus, Lactobacillus lactis,* and *Lactobacillus helveticus.*

3. The tablet of claim **1**, wherein said vitamin is selected from the group consisting of vitamin C and vitamin E.

4. The tablet of claim **3**, wherein said amino acid comprises a sulfur containing acid.

5. The tablet of claim **4**, wherein said amino acid comprises L-cystine.

6. The tablet of claim **1**, wherein said mineral salt is selected from the group consisting of the carbonates, oxides, and phosphates of calcium and magnesium suited for human ingestion.

7. The tablet of claim **6**, wherein said mineral salt comprises calcium carbonate, calcium phosphate, magnesium carbonate and magnesium oxide.

8. The tablet of claim **1**, wherein said means for stimulating bacterial growth is selected from the group consisting of autolyzed yeast extract, acid whey, and enzyme hydrolyzed casein.

9. The tablet of claim **1**, wherein said dietary fiber is present at a level corresponding to from about 320 mg to 510 mg per 750 mg total.

10. The tablet of claim **1**, wherein said amino acid is present at a level corresponding to from about 1 mg to 3 mg per 750 mg total.

11. The tablet of claim **1**, wherein said live bacteria are present at a level corresponding to from about 25 mg to 105 mg per 750 mg total.

12. The tablet of claim **1**, wherein said mineral salts are present at a level corresponding to from about 5 mg to 120 mg per 750 mg total.

13. The tablet of claim **1**, wherein said vitamin comprises vitamin C present at a level corresponding to from about 10 mg to 20 mg per 750 mg total.

14. The tablet of claim **1**, wherein said vitamin comprises vitamin E present at a level corresponding to from about 2 I.U. to 10 I.U. per 750 mg total.

15. The tablet of claim **1**, wherein said means for stimulating bacterial growth comprises autolyzed yeast extract present at a level from about 15 mg to 40 mg per 750 mg total.

16. The tablet of claim **1**, wherein said means for stimulating bacterial growth comprises acid whey powder present at a level from about 30 mg to 50 mg per 750 mg total.

17. The tablet of claim **1**, wherein said means for stimulating bacterial growth comprises enzyme hydrolyzed casein present at a level from about 4 mg to 5 mg per 750 mg total.

18. The tablet of claim **1**, further comprising lecithin.

19. A dietary fiber based vitamin, mineral, and beneficial bacteria tablet with enhanced bacterial viability, comprising: apple fiber, L-cystine, lecithin, acidophilus powder, propionic acid bacterium powder, *Leuconostoc citrovorum* powder, autolyzed yeast extract, acid whey powder, whey protein concentrate, vitamin C, vitamin E, a food grade alkaline salt of calcium, a food grade alkaline salt of magnesium, lactose, and enzyme digested casein.

* * * * *

EXHIBIT I

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Breton et al.
Appl. No.:	10/505,305
Conf. No.:	6006
Filed:	October 27, 2004
Title:	ORALLY ADMINISTRABLE COMPOSITION FOR THE PHOTOPROTECTION OF THE SKIN
Art Unit:	1651
Examiner:	I. Marx
Docket No.:	112701-434

AFFIDAVIT UNDER 37 C.F.R. § 1.132

Sir: Isabelle Bureau-Franz

I hereby state as follows:

1. My experience and qualifications are as follows:

__PharmD, PhD, research coordinator. Main Scientific objective was to study how nutritional supplementation can influence beneficial effect on skin.

2. I am one of the named inventors of the above-identified patent application and am therefore familiar with the inventions disclosed therein. I am also familiar with the study performed relating to the synergistic effects of the presently claimed composition and the summary of same attached as Exhibit B.

3. I have reviewed the outstanding Office Action dated December 20, 2007 pending against the above-identified patent application. In addition to considering the outstanding Office Action, I have reviewed the references cited therein as well as the pending claims.

4. The present invention is directed, in part, to an oral composition that includes an admixture of very specific constituents that surprisingly and unexpectedly elicit an enhanced synergistic effect or response in respect of the photoprotection of the skin. Specifically, the present disclosure relates, in part, to a composition for the photoprotection of the skin comprising a photoprotecting effective amount of at least one probiotic lactic acid bacterium, at least one carotenoid. and a yeast extract.

5. Although exposure to ultraviolet radiation may be necessary for humans to produce vitamin D, growing evidence suggests that extensive exposure to sun-light, in particular ultraviolet radiation, causes a variety of problems in the skin including, but not limited to, introduction of certain skin cancers and induction of accelerated skin aging. In addition to these established health concerns, research has also provided evidence suggesting that exposure to ultraviolet radiation may negatively affect a variety of immune responses in living beings both locally, within the UV-irradiated skin, and also systemically, *i.e.*, at sites distant from the irradiated skin.

6. With respect to the present disclosure, it has been surprisingly found that the admixture of a photoprotecting effective amount of at least one probiotic lactic acid bacterium, at least one carotenoid, and a yeast extract elicits an enhanced synergistic effect or response with respect to the photoprotection of the skin. The composition has been found to be effective not only for preventing inflammation or irritation of the skin after exposure to ultraviolet radiation, but it has also been found effective to provide complete prophylactic protection against the immunosuppressive effects of ultraviolet radiation. Specifically, the composition of the present disclosure is able to block or reduce the adverse clinical, histological and immunological effects of solar radiation exposure on the skin.

7. Attached hereto as Exhibit B, is a summary of a controlled study demonstrating the efficacy of the presently claimed composition with respect to the photoprotection of the skin. The study performed was a contact hypersensitivity (CHS) reaction test performed on female

hairless mice. The mice were divided into groups of ten, and four groups were used to complete one test round. Of the four groups in the test round, two groups were exposed to ultraviolet radiation (UVR) and two groups were not, as is demonstrated by the "No UVR" and "2.5 MED UVR" columns in Table 1 of Exhibit B. In each of the two different UVR exposure groups, one of the two groups therein was sensitized with the contact allergen 2,4-dinitroflurobenzene (DNFB), whereas the other group served as a control and was exposed only to acetone, as is demonstrated by the "Sensitized" and "Control" columns in Table 1 of Exhibit B.

8. Depending on the group being tested, the mice were fed a variety of formulas of food including a food with no additional supplements; a food with maltodextrin; a "matrix" food having beta-carotene, lycopene, inactivated yeast extract and excipients such as, for example, magnesium stearate, corn starch, and silicon dioxide; a food with carotenoids; and the "matrix" food that was also supplemented with a bacteria (La1). The formulas for the treatments are set forth in Table 1 of Exhibit B.

9. The mice received the respective treatments according to the scheme set out in Figure 1 of Exhibit B. The sensitization occurred about five or six days after UVR exposure by painting either DNFB or acetone on the abdomen of the animal. A challenge was performed on day 12 of the study, after UVR exposure, by painting DNFB on the right ear of the mouse and acetone on the left ear of the mouse. Figure 2 demonstrates the results of the challenge and the data is expressed as the difference between the swelling of the right ears and left ears. Blood and skin biopsies were taken at necropsy for analysis of IL-10 serum levels and epidermal LC density.

10. As is illustrated by Figure 2 of Exhibit B, the control test without UVR exposure (column 2) and the composition according to the present disclosure and having a photoprotecting effective amount of at least one probiotic lactic acid bacterium, at least one carotenoid, and a yeast extract (column 3) showed the greatest immunological response to the allergen, as is demonstrated by the larger differences between the swelling of the right and left ears of the mice. The increased amount of swelling of the right ear of the mice tested with respect to columns 2

and 3 indicates that the skin reacted readily to the presence of the allergen on the right ear. In other words, the skin reacted readily to the presence of the allergen on the right ear because the animal did not experience local immunosuppression due to exposure to UVR.

11. As is also illustrated by Figure 2 of Exhibit B, the control test plus exposure to UVR (column 1), the "matrix" formula alone plus exposure to UVR (column 4), and the carotenoids alone plus exposure to UVR (column 5) all failed to block or reduce the clinical, histological and immunological effect of UVR exposure of the skin of the animal. This is clearly demonstrated by the decreased amount of swelling of the right ear of the animal, which indicates that the immune system of the animal was not acting efficiently in response to the presence of the allergen after exposure to UVR. In other words, the formulas corresponding with the data of columns 1 and 4-5 proved unsuccessful in preventing local immunosuppression resulting from UVR exposure.

12. With respect to the cited references, the skilled artisan would have no reason to combine the cited references to obtain the present claims because the cited references are directed to unrelated products that have completely different objectives and fail to even recognize the surprising and unexpected effect that the specific composition has on the photoprotection of the skin, as is clearly demonstrated by the results of the study attached as Exhibit B.

13. *Vesely* is directed toward a beverage containing live bacteria that is used to increase, balance and supplement intestinal flora. See, *Vesely*, col. 3, [0016]. *Shields* is entirely directed toward canine food formulations that optimize digestibility of nutrients in specific canine breeds. See, *Shields*, column 3, lines 30-36. *Runge* is entirely directed toward dry microorganism cultures and the processes for producing same. See, *Runge*, Abstract. *Berggren* is entirely directed toward a sports drink that is designed to increase the energy and fluid levels in an individual, as well as reduce stress. See, *Berggren*, page 2, line 39-page 3, line 4. *Brassart* is entirely directed toward a biologically pure culture of a lactic acid bacterium strain. See, *Brassart*, Summary of the Invention. *Reddy* is entirely directed toward a supplement that permits

the longevity of certain health promoting bacteria in tablets. See, *Reddy*, column 1, lines 10-20. As such, there is absolutely no guidance in the cited references for one of skill in the art to choose the active agents and amount of agents present in the instant claims to achieve the unexpectedly improved photoprotective effect on the skin as Applicants have demonstrated in the present disclosure and in the summary of the study attached as Exhibit B.

14. In contrast to the presently claimed subject matter, the cited references are completely unconcerned with blocking or reducing the adverse clinical, histological and immunological effects of solar radiation exposure on the skin, as demonstrated above and in Exhibit B. Consequently, the skilled artisan would have no reason to combine the cited references to arrive at a photoprotecting composition in accordance with the present claims, nor would the skilled artisan have any reasonable expectation of success in combining the cited references.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

Date: April, 8th, 2008

Print Name Isabelle Bureau-Franz

EXHIBIT B

METHODS AND MATERIALS

This study was conducted by Nestec S.A. at the Centre International de Toxicologie (CIT) in Evreux, France.

The study consisted of a contact hypersensitivity (CHS) reaction test in female hairless Skh:hr1 mice. Ten mice were randomly assigned to each treatment group. One test consisted of four treatment groups, as is demonstrated by the rows of Table 1 below. Two of the four treatment groups were not exposed to any ultraviolet radiation (UVR), and two of the four treatments groups were exposed to 2.5 MED solar-simulated UVR, as is demonstrated by the designations "No UVR" and "2.5 MED UVR" in Table 1 below. In each of the two different UVR exposure groups, one of the two groups therein was sensitized with the contact allergen 2,4-dinitroflurobenzene (DNFB), whereas the other group served as a control and was exposed only to acetone, as is demonstrated by the "Sensitized" and "Control" columns in Table 1 below.

Table 1
Formulations Tested

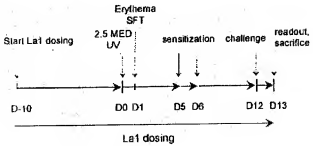
Supplementation	No UVR		2.5 MED UVR	
	Control	Sensitized	Control	Sensitized
No treatment	Group 1	Group 2	Group 3	Group 4
Maltodextrin	Group 5	Group 6	Group 7	Group 8
Matrix*	Group 27	Group 28	Group 29	Group 30
Carotenoids	Group 31	Group 32	Group 33	Group 34
Matrix* + La1 10^8 cfu/d**	Group 35	Group 36	Group 37	Group 38

* The matrix consisted of beta-carotene, lycopene, inactivated yeast extract, and excipients (magnesium stearate, corn starch and silicon dioxide). The carotenoid preparation was composed as the matrix without the yeast extract.
** Bacterial inactivation was by γ-irradiation at 41 kGy (Studer AG,Däniken).

The matrix components for the study were from SIIT, Italy. The formulations were mixed under nitrogen and packed under an inert atmosphere, shipped on dry ice and stored at 4°C.

The animals received the respective treatments according to the treatment scheme shown below in Figure 1.

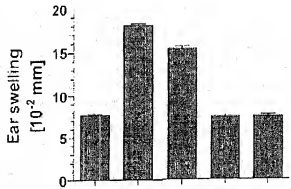
Figure 1
Animal Treatment Scheme



The sensitization occurred five and six days after UVR exposure by painting either DNFB or acetone on the abdomen of the mice. A challenge was performed on day 12 after UVR exposure by painting DNFB on the right ears and acetone on the left ears of the animals. The data of Figure 2 below is expressed as the difference between right and left ear swelling, measured in millimeters. Blood and skin biopsies were also taken at necropsy for analysis of IL-10 serum levels and epidermal LC density.

RESULTS AND CONCLUSIONS

Figure 2
Results of the Tests



Column 1 - Control +UV
Column 2 - **Control without UV**
Column 3 - Matrix + La1 10^8 live + UV = **composition according to the invention**
Column 4 - Matrix alone +UV
Column 5 - Carotenoids alone +UV

Figure 1 demonstrates the lack of a photoprotective effect of the matrix (carotenoids and yeast) alone, and carotenoids alone, as is shown by columns 4 and 5. Figure 1 also demonstrates the effect of La1 plus the matrix on CHS reaction, as is shown by column 3. The data resulting from the presence of La1 10^8 cfu in the matrix was significantly different than the data resulting from the control +UV ($p<0.001$).

Thus, it has been shown that the matrix alone (column 4) or the carotenoids alone (column 5) had no photoprotective effect on UVR-induced immunosuppression ($p>0.05$ relative to control +UV). Instead, a photoprotective effect on UVR-induced immunosuppression was observed only for the tests involving the matrix (carotenoids and yeast) plus La1.